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**Biochemical basis of the effect of pre-slaughter stress
and post-slaughter processing conditions
on meat tenderness**

A thesis submitted in partial fulfilment
of the requirements for the degree
of
Master of Science – Biochemistry
at
Lincoln University

by

Friday Osas Obanor

LINCOLN UNIVERSITY
2002

An abstract of a thesis submitted in partial fulfilment of the requirements for the degree of
Master of Science (M. Sc) Biochemistry

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Previous research has shown that pre-slaughter stress of cattle reduces beef quality because of its effect on post mortem muscle pH. Spray washing in the cattle yard is a hygiene requirement of the New Zealand Ministry of Agriculture and Fisheries (MAF) in order to reduce the contamination of carcasses. However, the washing stresses the animal. This study investigated the effect of spray-washing cattle on beef quality. Spray washing of cattle resulted in considerably more variation in the ultimate pH (pH_u) of the meat (5.52 to 6.48) compared to meat from dry animals (5.50 to 5.79). The increased variability in pH_u was reflected in the meat shear force values from the spray-washed animals, which ranged from 5.2 to 14.5 kgF, while that from the dry animals ranged between 4.8 and 8.6 kgF. There was a tendency for meat from spray-washed animals to be tougher than meat from dry animals ($p = 0.089$). The effect of spray washing on calpain I autolysis, desmin and troponin T degradation during post mortem period was determined. Results showed that spray washing tended to cause an increase ($p = 0.07$) in post mortem degradation of desmin but had no significant effect on troponin T degradation or calpain I autolysis.

The second part of the research investigated the effect of post-slaughter processing conditions on turkey breast meat tenderness, and the biochemical basis for the toughening of turkey breast meat. Anecdotal evidence showed that consumers perceived turkey breast meat as being tough. The preliminary results of this research indicated that the tenderness of turkey breast meat was variable and the mean shear force was higher than would be acceptable to consumers. The observed toughening of turkey breast meat may be a result of post-slaughter treatments of turkey carcasses, such as the rate of carcass cooling, stunning method and carcass deboning time. The effect of these factors on turkey breast meat tenderness were investigated in this research. Post-slaughter temperature and pH of turkey

breast muscle was measured at different times. The carcass temperature ranged from 32°C to 36°C at 15 minutes post slaughter. Immersion chilling (1st water bath and 2nd ice slush bath) rapidly reduced the internal breast muscle temperature to about 10°C by 75 min after stunning. In all three experiments (2, 3 & 4), cold blast treatment significantly decreased the internal breast muscle temperature compared to the breast muscle that was chilled only. The breast muscle temperature immediately after a cold blast treatment (about six hours post-slaughter) ranged from 2-6°C. In this study, the mean pH of turkey breast muscle at 15 min post-slaughter was 6.52, indicating that turkey breast muscle exhibited accelerated rigor mortis compared to lamb or beef. The pH of turkey breast muscle at six hours post-slaughter was close to the ultimate pH, which ranged from 5.80 to 5.96.

Results of this research showed that turkey processing conditions have a toughening effect on the resulting breast meat. Deboning of turkey breast muscle three hours post-slaughter caused between a 80% and 90% increase in toughness of the meat compared to muscle aged on the bone for 24 hours. The use of a blast freezer to rapidly cool the breast also toughened the meat by an average of 30%. The mechanisms responsible for the meat toughening were both muscle contraction and a reduction of proteolysis. The sarcomere length of muscles deboned three hours post mortem was significantly shorter than muscles deboned at 24 hours post mortem. The myofibrillar fragmentation index (MFI), which is a measure of proteolysis, was significantly higher for muscles aged on bone for at least 24 hours compared to muscles deboned three hours post-slaughter. It is known that meat tenderness improves during post mortem ageing. Results of experiment 4 showed that six days extended ageing of pre-rigor or post-rigor deboned turkey breast muscle significantly improved the meat tenderness. In both muscles deboned at 3 h and 24 h post mortem, there was a 25% decrease in the shear force values after the 6-day ageing period. During the ageing period, MFI increased significantly both for muscles deboned at three hours post-slaughter ($p = 0.025$) and for the muscles aged on bone for 24 hours prior to deboning ($p = 0.018$).

Activities of calpain I, II and calpastatin were measured at three different times during post-slaughter ageing (0, 3 and 24 hours) in muscles aged on bone for 24 hours and muscles deboned three hours post-slaughter and then, aged for 24 hours. The activity of calpain I rapidly declined with increasing post mortem time to the extent that only trace levels were

detected at three hours, and no calpain activity was detected at 24 hours post mortem. In contrast, there was no loss in calpain II activity over the post mortem period. Calpastatin retained only 35% of its at-death activity in turkey breast muscle at three hours post-slaughter and there was no calpastatin activity at 24 hours post-slaughter. Furthermore, deboning time had no significant effect on the activity of calpain I, II and calpastatin at 24 hours post mortem.

Based on these results, it can be concluded that the observed toughening of turkey breast meat originates from the rapid cooling of turkey carcasses and the three hours post slaughter deboning time. The mechanism of the toughening of turkey breast muscle deboned three hours post mortem is likely to be a direct result of cold shortening rather than reduced calpain activity. This suggests that the reduced proteolysis as measured by MFI is a result of reduced accessibility of the calpain to myofibrillar protein because of the shortened sarcomere length of muscles deboned at three hours post slaughter and then aged for 24 hours.

Keywords: Beef, turkey, stress, tenderness, sarcomere length, MFI, proteolysis, calpain, calpastatin, debone and chilling.

ACKNOWLEDGEMENTS

This thesis was made possible by the generous help and support of many people, to whom I am deeply indebted.

I would like to thank my supervisor, Dr Jim Morton who provided ongoing assistance, encouragement and direction throughout the duration of this work. Thanks a million for spending your valuable time to organize and proof read my drafts, and for allowing yourself to be easily accessible. I am also very grateful to my associate supervisor, Professor Roy Bickerstaffe, who provided me with support in the experimental work and also assisted in proof reading my drafts. Thank you too, to Dr Geert Geesink, for his technical assistance on the biochemical techniques used in this work.

My special gratitude goes to Bradley Stevenson, not only for his advice on gel electrophoresis, but also for his wonderful company during my long hours of work.

I am indeed grateful to my 'small boss,' Alaa El-Din for teaching me how to determine meat tenderness and for assisting me during sampling. Your encouragement lifted me up when I felt gloomy. Many thanks to other members of the group, including Dr Mohammed Ilian, Meegalla Chandratne, Lucinda, Karl, Matthew and Grant, all of you made my study years so pleasurable. I know that without you, it would have been a nightmare.

I would also like to take this opportunity to thank my partner, Astrid, for her love, support and encouragement throughout the duration of this research. In addition, I would like to thank her for helping to type part of this work and for her toleration of my moodiness after a hard day. A special thanks too, to my friends, Anne and Charles Addei who rescued me when I was 'drowning'.

To my fellow 'inmates,' Thayalini, Fred, Tamanna, Boyd, Manfred and Li, I would like to say thank you all for your wonderful jokes and enthusiasm. I appreciate the pleasant atmosphere you all provided throughout the duration of my study. Remember: 'Don't work too hard, take it easy'.

I also wish to pass on my deepest gratitude to Monika Walter for her invaluable advice, kindness, and honesty. It was a pleasure working with you. My thanks also go to Nick and Fleur for their wonderful sense of humour. You are all great people.

Finally, I would like to say thank you to the administrative secretary, Sue Winter, for her warm reception, helpfulness and quick sense of humour.

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Chapter 1

1.0 Introduction

Tenderness is one of the most important factors in determining the acceptability of meat, particularly beef (Savell et al., 1987; Boleman et al., 1997). Tenderness is an organoleptic quality which is difficult to define physically. However, tenderness involves the ability of meat to be sheared, compressed, and ground during consumption and therefore, depends on the mechanical properties of the muscles. Consumers perceive meat toughness and tenderness as the ease with which meat structure is disorganised during mastication (Lepetit and Culioli, 1994).

Meat tenderness strongly influences the re-purchase intent of consumers and their choice of particular cuts of beef (Boleman et al., 1997). Consumers replace products that fail to meet their quality expectations, as well as products that have inconsistent quality. In addition, Chrystall et al (1994) noted that the tenderness of the meat is an important determinant of the price consumers are expected or willing to pay for a particular cut of beef. Consumers are willing to pay a premium for meat that is very tender. In a survey on beef tenderness conducted by Morgan et al (1991) in Australia, the authors found that beef was unacceptably variable in tenderness, and that inconsistency in tenderness was identified as one major problem that faces the beef industry. This factor has been thought to contribute largely to the decline in the demand for beef. Thus, solving the problem of inconsistent meat tenderness is a top priority of the meat industry.

Meat toughness/tenderness has been the subject of extensive scientific research. One of the problems encountered in the quest for a possible solution to meat toughness is converting consumers' expectations into measurable parameters. One such technique is the use of sensory panels to assess the tenderness of meat. Although this methodology has been very important to the improvement of beef (Shackelford et al, 1995), it has been less effective in differentiating subtle effects on toughness. A physical technique that has proved useful in assessing the tenderness of meat is the resistance of meat to shear. This method is often used in parallel with consumer sensory panelling methods. Results from such studies in New Zealand showed that consumers' perceived beef and lamb shear force value of less

than 8 kgF (as assessed by the MIRINZ tenderometer) as being tender. In contrast, meat with shear force value above 11 kgF is considered tough and unacceptable to the consumers (Table 1.1) (Bickerstaffe et al, 2001).

Table 1.1: Consumer perceptions of the tenderness of beef and lamb loins and their shear forces.

Tenderness classification for consumers	Shear force (kgF) range
Very tender	< 5.0
Tender	5 – 7.9
Acceptable	8 – 10.9
Tough	11.0 – 14.9
Very tough	≥ 15.0

Meat tenderness is highly influenced by ante-mortem and post-mortem factors (Asghar and Pearson, 1980; Ouali, 1990). Ante-mortem factors that influence meat tenderness include animal breed, animal husbandry practices, handling and pre-slaughter stress. Physiological stressors such as feed withdrawal and spray washing may contribute to meat toughness. Cattle spray washing is a hygiene requirement, which may lead to stress on the animal. There is no information available on the possible effects of spray washing on beef quality in the literature. Such information would be useful to meat processors, as spray washing may be a source of variability in beef quality. Hence, the first part of this research will investigate the effects of spray washing on beef quality.

A number of post-mortem factors can influence meat quality (Figure 1.1). These factors include, methods of stunning and slaughtering, application of electrical stimulation, deboning time, chilling method, environmental temperature and extent of post-mortem ageing. Although these processing conditions have received wide research attention in beef, lamb, pork and chicken, little information is available on the effects of these conditions on turkey meat quality, particularly the tenderness.

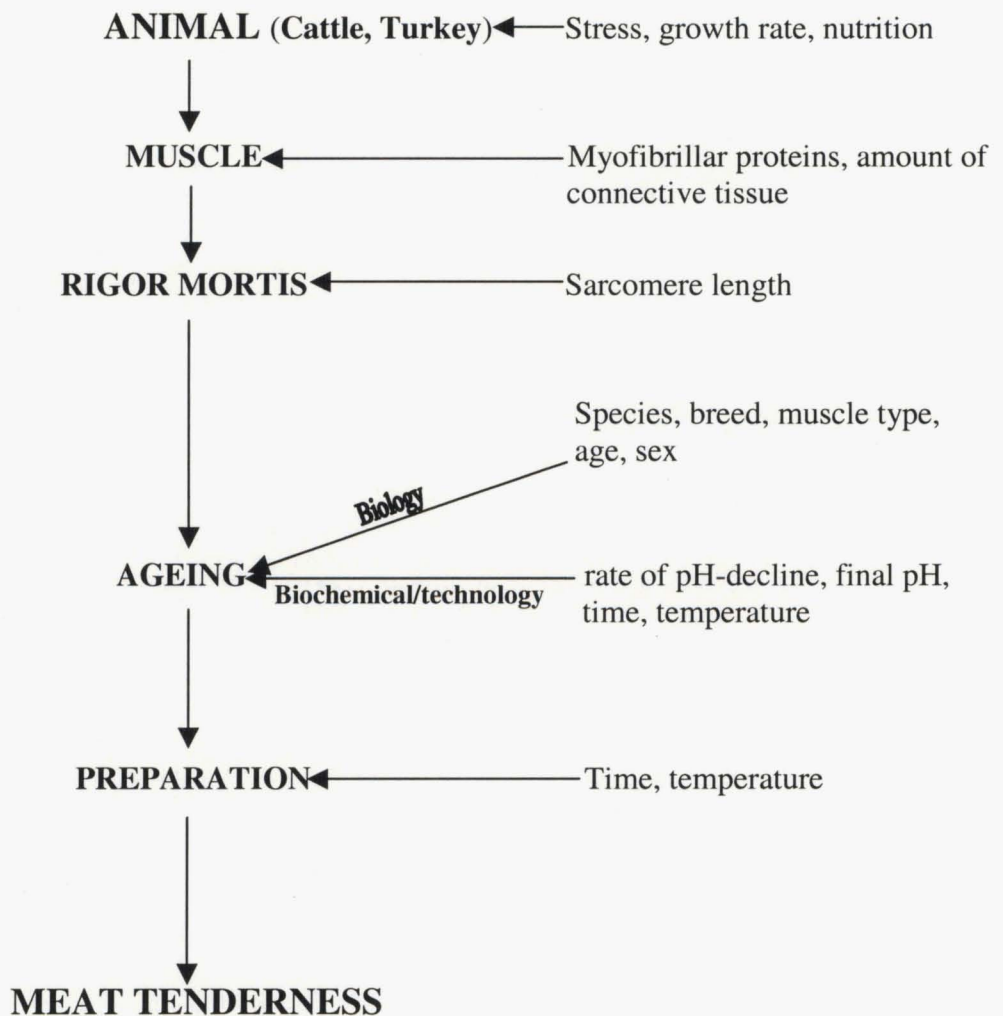


Figure 1.1: Factors influencing meat tenderness (Adapted from Geesink, 1993).

Previous research in turkey meat quality has focused mainly on the mechanisms involved in pale, soft and exudative muscles (PSE) syndrome. Pale, soft and exudative muscle is

characterized by meat with pale colour, soft texture, and poor water-holding capacity (Sosnicki and Wilson, 1991). This quality defect in turkey breast meat is associated with prolonged high post-mortem temperatures ($> 37^{\circ}\text{C}$), a rapid rate of post-mortem glycolysis and an early onset of rigor mortis (McKee and Sams, 1998). To reduce the incidence of PSE, processors utilize a rapid chilling procedure, which ensures a reduced rate of glycolysis and delays the onset of rigor mortis. Rapid post-slaughter chilling of turkey carcasses may, however, be a source of variation in turkey meat tenderness.

Over the last decade, the New Zealand turkey industry had seen increased growth in turkey meat production, although according to an unpublished industry report consumers are concerned with inconsistencies in the tenderness of turkey breast meat. In order to restore consumers' confidence, any variability in turkey meat tenderness must be solved. Variability in turkey meat tenderness may be a result of post-slaughter processing conditions on turkey carcasses. In New Zealand, the effect of the existing processing procedure (Figure 5.1) on turkey meat tenderness has not been documented. For example, the effect of rapid chilling of turkey carcasses on the resulting meat tenderness has not been investigated. In addition, there is little or no information on the effect of the post-mortem deboning time and the extent of ageing on the tenderness of turkey breast meat. The availability of such information will allow turkey processors to produce consistent meat quality and restore consumers' confidence. Thus, turkey meat would be able to compete favourably in domestic and global markets.

The main aims of this research were to investigate the influence of post-slaughter processing conditions and the biochemical basis for the variation in turkey breast meat tenderness. The existing turkey processing procedure would be optimised in order for the processors to produce consistently tender meat. To achieve these objectives, five experiments were performed. Experiment 1 was designed to investigate whether there was any toughening in turkey breast meat. Because there was evidence of variability in turkey breast meat tenderness in experiment 1, the specific objectives of experiment 2, were to investigate the effect of rapid chilling and post-slaughter deboning time of turkey breast muscle on the tenderness of the resulting meat. In the third experiment, the influence of electrical stunning, and extent of cooling by chiller and cold-blast treatments was determined. Experiment 4 investigated the existing processing procedure (as described in

section 5.1) against a new method of 'on bone' ageing (ie ageing muscles on bone for at least 24 h before deboning) on the tenderness of turkey breast meat. The final experiment was designed to determine whether the toughness of turkey breast was a result of reduced activity of the enzyme system (calpain) implicated in post-mortem meat tenderisation.

Chapter 2

Literature Review

2.1 Introduction

Skeletal muscle is a tissue that performs the function of locomotion in higher animals. This tissue is also a source of food for man when it is known as meat. The conversion of muscle to meat involves complex biochemical, physical and structural changes (Lawrie, 1991). At death, the blood supply to the muscle of an animal ceases and, as a consequence, there is no supply of oxygen to the muscles. As a result, the muscle cell switches to anaerobic oxidation which causes stored glycogen to be converted, through anaerobic glycolysis, to lactic acid. The accumulation of lactic acid causes a decline in muscle pH, which results in structural proteins becoming liable to denaturation and protein degradation (Lawrie, 1991). Other changes that occur in post-mortem muscle include a decline in muscle ATP levels, formation of actomyosin and the inextensibility of muscle fibres.

In this chapter, the structure of the skeletal muscle will be described briefly. Then, the biochemical and biophysical changes in post-mortem muscle and their effects on meat tenderness will be discussed. In addition, the effects of pre- and post-mortem treatments of muscles on the resulting meat tenderness are reviewed.

2.2 Muscle Structure

The skeletal muscle consists mainly of long, thin muscle fibres, each of which is a single multinucleate cell. Nearly 60% of the volume of each fibre cell consists of myofibrils. These are long, cylindrical cytoskeletal structures that transverse the length of the cell. The myofibrils are organised into repeating subunits, called sarcomeres, that are visible under a light microscope (Harper, 1999). A sarcomere is the longitudinal segment of a myofibril between crosswise divisions known as Z-bands (Figure 2.1). The sarcomere is a contractile unit of the myofibril and consists mainly of thick and thin filaments. The myosin and actin

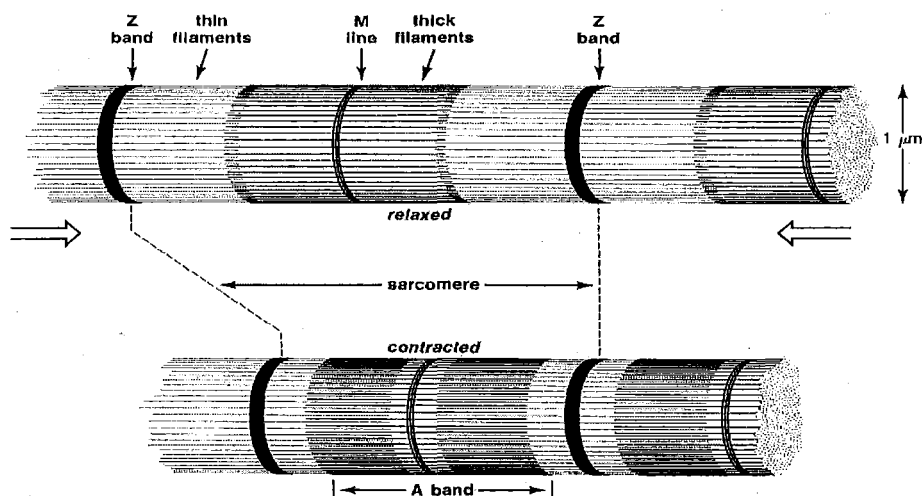


Figure 2.1: Structure of striated muscle showing a relaxed and contracted sarcomere (McGilvery and Goldstein, 1983).

molecules, which are key components of the thick and thin filaments respectively, make up most of the myofibrillar proteins. The thin filament (actin) is attached to each side of the Z-band, which are rigid thin circular plates of protein. The gaps between the thin filaments are filled with the thick filaments (myosin), which intermesh end to end with the thin filament bundles. Myosin is held in a bundle by a protein in the region known as the M-line (McGilvery and Goldstein, 1983).

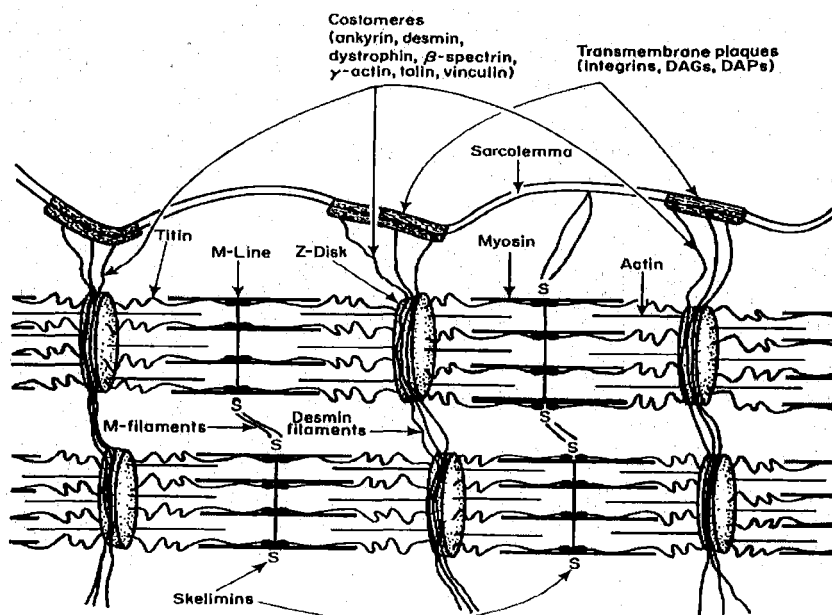


Figure 2.2: Schematic diagram showing the structure and protein composition of costameres in striated muscle relative to Z-disks and the myofibrillar lattice (Taylor et al, 1995).

The framework of the myofibrillar system is strengthened and held in position by the cytoskeletal proteins. These proteins include nebulin, troponin T, desmin, titin, vinculin and actinin. Desmin makes up the intermediate filaments that interconnect the myofibrils into a continuous lattice in the cell (Harper, 1999). Titin, a very large molecular weight protein, is a constituent of the gap filaments (and runs from the M-line to about the middle of the I-band), while nebulin starts at the Z-line (Wang and Wright, 1988). Myofibrils are linked to the cell membrane (sarcolemma) in the I-band region via the costamers, which contain the protein vinculin (Figure 2.2)

Each fibre cell is ensheathed by a connective tissue called endomysium (Figure 2.3). At the macroscopic level, a connective tissue called perimysium surround bundles of muscle fibres. The muscle as a whole is ensheathed by another layer of connective tissue called the epimysium, which joins with the tendons that attach the muscles to the skeleton (Tornberg, 1996).

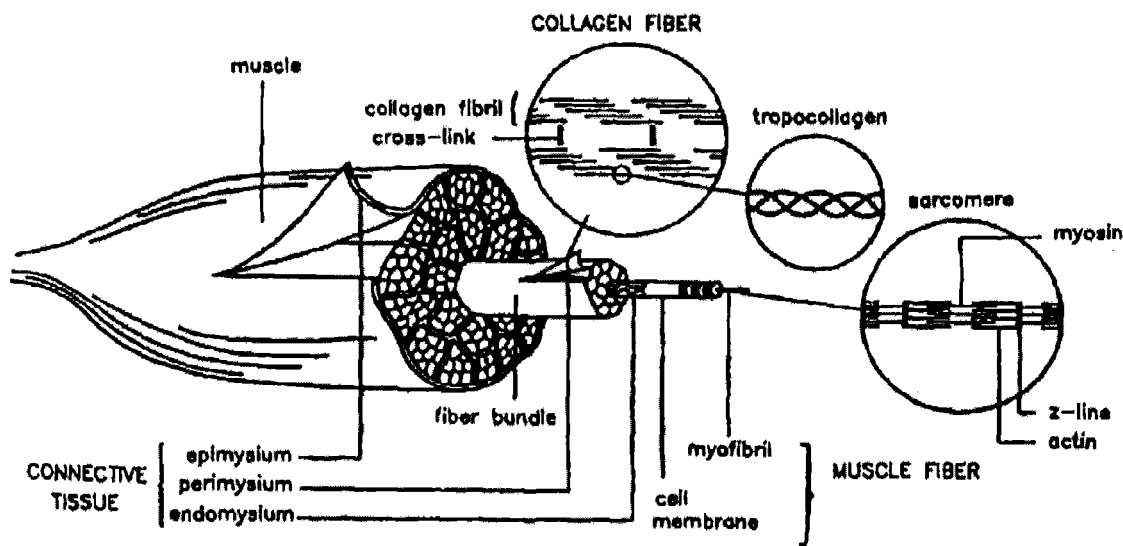


Figure 2.3: The structural hierarchy of a muscle (Tornberg, 1996).

Vertebrate skeletal muscles have broadly similar structural properties. However, muscles vary from each other. Some of their properties such as visual appearance and their biochemical and physiological characteristics reflect the properties of the fibre types present in the muscle (Swan, 1993; Karlsson et al., 1999). Muscle fibres can be divided into three broad groups, fast-twitch (white), slow-twitch (red) and intermediate, on the basis of histochemical staining or molecular techniques (Harper, 1999). Muscle fibre types contain

different concentrations of myoglobin, oxidative and glycolytic enzymes and different contractile proteins. The red fibres contain more myoglobin than white fibres. The composition of a muscle is adapted to its function in the body. For instance, muscles that are involved in posture have a higher oxidative than glycolytic capacity, whereas muscles involved in locomotion have a higher glycolytic than oxidative capacity (Essen-Gustavsson, 1996). Hence, muscles involved in posture contain more red than white fibres, while those muscles involved in locomotion contain a higher proportion of white fibres. For example, migratory birds such as ducks have mostly red fibres in their flight muscles, whereas ground-living birds such as chickens and turkeys have mostly white fibres.

2.3 Post-mortem muscle metabolism

The primary function of skeletal muscle is to generate contractile forces that are expressed through the limbs as voluntary movement (Harper, 1999). Muscles utilise ATP as the major source of energy in order to perform muscular functions and to maintain functional integrity. In post-mortem muscles, the ATP splitting enzyme that has the highest potential ATPase activity, is myosin ATPase. In a resting muscle, there is a continuous breakdown of ATP to ADP and inorganic phosphate. Because of the large concentration of myosin, this ATPase activity may well be the major ATP depletion mechanism (Greaser, 1986). Another enzyme that contributes to ATP hydrolysis in post-mortem muscles is the sarcoplasmic reticulum Ca^{2+} -ATPase. The function of this enzyme is to move calcium out of the cytosol during the rest cycle of muscle contraction in an ATP-dependent process. Because the muscle cells attempt to keep the calcium concentration in the cytosol low, this enzyme functions continuously post-mortem until ATP is depleted.

When there is a supply of oxygen to the mitochondria, mitochondrial ATPase catalyses the synthesis of ATP from ADP and inorganic phosphate (Warriss, 2000). But when the oxygen supply ceases, as in post-mortem muscles, the mitochondrial ATPase will hydrolyse ATP instead of synthesising it. This ATPase activity has been shown to account for a major part of ATP turnover in heart cells (Haworth et al, 1981). Other enzymes such as glycogen phosphorylases and phosphofructokinase, which require ATP for their action, should also continue to function (Suwidji and Yamanaka, 1998).

2.3.1 Glycolysis and Muscle pH

Muscles begin to undergo physical and chemical changes immediately post-slaughter. These changes are dependent on the environmental temperature, relative humidity and the activities of endogenous enzymes. In a living muscle, ATP is generated by mitochondrial oxidative metabolism or by anaerobic glycolysis. During the post-mortem period, the blood supply to muscles ceases, and since there is no longer a source of oxygen, the synthesis of mitochondrial ATP is stopped. Thus, post-mortem muscle metabolism is fuelled by anaerobic glycolysis (O'Halloran et al., 1997; Warriss, 2000). The end product of these reactions is lactic acid, which accumulates over the post-mortem time period (Ozawa et al., 1990) and contributes to the fall in muscle pH from about 7.2 to around 5.5 (Martin et al., 1983; Warriss, 2000).

After death, the muscle glycogen reserve is mobilised because there is no new supply of glucose. Available glycogen will be degraded until it is no longer accessible to glycogen phosphorylase or until low pH inactivates the glycolytic enzymes. It should be emphasised, however, that in rested animals not all glycogen reserves are depleted even after 36 days post-mortem (Greaser, 1986). Tarrant and Mothersill (1977) reported that there was significant variation in muscle glycolysis between animals and between muscle locations within a beef carcass. The amount of glycogen remaining when glycolysis ceases varies from animal to animal and muscle to muscle (Lawrie, 1992). Also, the rate of depletion of muscle glycogen post-mortem depends on the animal species (Severini et al, 1989). Glycogen reserves decline more rapidly in pig (Bendall, 1973) and avian muscles (chicken and turkey) than beef or lamb muscles. For example, Bendall (1973) found that the concentration of glycogen in pig muscle declined by 30 - 50% within 10 minutes after death, whereas in beef LD, glycogen levels were still at 84% of the initial concentration at 24 hours post-mortem.

Muscle pH falls after death because of the conversion of muscle glycogen to lactic acid. The rate of pH fall depends on the initial concentration of glycogen, the rate of glycolysis, animal species, pre-slaughter treatment (type of stress and intensity of the stress imposed on animals) and storage temperature (Warriss, 2000). The post-mortem pH decline is linked to glycogen depletion with the pH of pig muscle declining more rapidly than lamb or beef (Bendall, 1973). The pH decline in turkey or chicken breast muscle is similar to that of pig

muscle. Dransfield (1994) showed that the acidification process usually takes 4–8 hours in pigs, turkey and chicken, 12–24 hours in sheep and 15–36 hours in cattle. Recent studies have also indicated that there is a large variation in the rate of pH fall between the slow, intermediate and fast glycolysing beef LD muscles (O’Halloran et al, 1997) (Figure 2.4).

Post-mortem muscle temperature can influence the rate of pH decline. Studies by Tornberg et al (2000) showed that in beef, at 20°C, a pH of 5.6 was attained by 4–5 hours and if chilled to 12°C, a pH of 5.6 was attained by 12–20 hours.

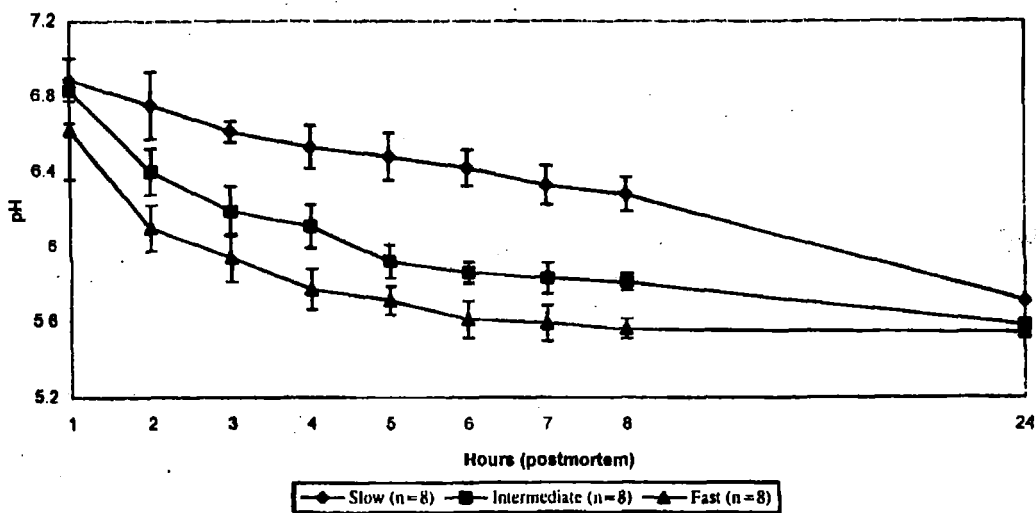


Figure 2.4: The pH decline of bovine *m. longissimus dorsi* during the first 24 h post-slaughter (O’Halloran et al, 1997).

2.3.2 Rigor Mortis

One of the most significant changes in muscle post-mortem is its transformation from being soft, pliable and stretchable, to a more rigid and inextensible state. This muscle state is known as rigor. It has been shown that the extensibility of muscles is directly related to the post-mortem loss of ATP (Peitrzak et al, 1997). Rigor mortis occurs when the ATP level falls below the very low level of 5 mM required to maintain relaxation (Warriss, 2000). At this low ATP concentration, actin and myosin molecules combine to form an actomyosin complex, which is irreversible (Goll et al, 1995).

Rigor mortis can be classed into three stages – delay, rapid and post-rigor (Briskey et al, 1962). The delay stage is that period of time in which there is no change in muscle

elasticity. The rapid stage begins when the muscle extensibility begins to decline and ends when the extensibility reaches its minimum. The post-rigor stage is the period after the rapid phase is completed (Greaser, 1986). The length of time of the various rigor stages is dependent on the animal species, type of muscle, storage temperature and pre-slaughter treatments.

Factors affecting the level of glycogen and creatine phosphate at death and the rate of post-mortem metabolism will determine the onset of rigor mortis (Warriss, 2000). The onset of rigor occurs when about 60% of the ATP is utilised. At 1°C, full rigor is reached by the time the pH has fallen to about 6.2 (Newbold, 1966). A fast glycolyzing muscle will develop rigor earlier than a slow one. Etherington et al (1987) showed that the development of rigor in chicken is around four hours post-mortem whilst in beef it can take up to 24 hours. Previous research has shown that turkey breast is a fast glycolyzing muscle (Pietrzak et al., 1997; Rathgeber et al, 1999). Therefore, rigor mortis in turkey should be complete within the first four hours post-slaughter.

Rigor mortis is associated with sarcomere shortening. Sarcomeres tend to shorten during the onset of rigor mortis, and to different extents in different muscles by a mechanism that has not yet been elucidated (Dransfield, 1992). Muscles that are stretched by being left on the carcass are restrained from shortening over the post-mortem period. Harris and Shorthose (1988) reported that in beef carcasses hung by the achilles tendon, the *semitendinosus* (ST) muscle is stretched and, consequently, the sarcomeres failed to shorten, whereas the *longissimus dorsi* (LD) muscle which was not under tension had shortened sarcomeres. That is, muscles will shorten if they are unrestrained as they enter rigor or they will develop tension if the muscles are maintained at a constant length (Devine et al, 1999).

Post-slaughter temperature affects the extent of rigor mortis. Nuss and Wolfe (1981) showed that the development of tension in beef muscles was maximal at 1°C or 2°C, to a lesser extent at 37°C, and the lowest at 16°C or 25°C. The shortening of muscles at low temperatures occurs by a different mechanism from that at higher temperatures. Locker and Hagyard (1963) reported that muscles removed from a beef carcass immediately post-mortem would shorten up to 40% at 2°C. This phenomenon is called cold shortening and is

quite different from rigor shortening. Cold shortening occurs rapidly if muscles have a low temperature, high pH value (>6.0) and ATP concentration of 5–6 mM. Rigor shortening develops more slowly (it can take many hours) when muscles have a low pH (<6.0) and an ATP concentration of <1 mM (Bendall, 1975). Muscles which have cold-shortened will undergo a second contraction at the time of rapid ATP decline (rigor shortening). This is expressed either as tension or shortening (Bendall, 1975). Cold shortening is more pronounced in muscles with substantial amounts of red muscle fibres compared to white muscle fibres (Bendall, 1975). The extent of cold shortening is, consequently, much less in rabbit, pig and avian muscles compared to beef muscles.

2.3.3 Ultimate pH

Ultimate pH (pH_u) is an important indicator of meat quality, and it is correlated with the tenderness, colour, water-holding capacity and shelf life of meat (Pearson and Young, 1989). A lower pH_u (<5.8) is associated with a high drip loss in turkey and broiler breast muscles (Barbut, 1997). In beef, a pH_u of 5.5 seems to be a prerequisite for tenderness (Watanabe et al, 1996; Purchas et al, 1999). If we can control meat pH_u , we can reduce the variation in meat quality (Van Laack et al, 2001). Inconsistency in meat quality costs the U.S. meat and poultry industries millions of dollars annually (Kauffman, 1996; Cannon et al, 1996). To improve consistency, we need to understand the factors that determine pH_u . Both intrinsic (species, animal age, type of muscle and position of the muscle) and extrinsic (pre-slaughter stress, slaughter conditions, post-slaughter handling and temperature) factors affect the extent of post-mortem glycolysis (Swan, 1993) and, as a consequence, ultimate pH. Of these, no single factor can explain more than 50% of the variation in pH_u (Van Laack, 2001). Thus, inconsistencies in meat quality due to variation in pH_u will continue to pose a problem to meat processors until the exact relationship between all the factors is understood. Some of the factors that may influence pH_u are discussed below.

2.3.3.1 Stress, glycogen concentration and glycolytic potential

Glycogen is the substrate for energy production. During the first day post-mortem, glycogen is converted into lactic acid and energy. The formation of lactate causes the decline in post-mortem pH (Greaser, 1986). Thus, the concentration of muscle glycogen, when the animal is slaughtered, will influence pH_u . For example, ante-mortem muscles

with low levels of glycogen will produce less lactic acid during post-mortem anaerobic glycolysis and, as a consequence, the meat has a high pH (McVeigh and Tarrant, 1982). Stress in animals initiates the secretion of adrenaline and noradrenaline that mobilises muscle glycogen reserves. Animals that respond actively to a stressor will tend to deplete muscle glycogen levels. The extent of muscle glycogen depletion depends on the type and intensity of the stressor. Stress, such as feed withdrawal, which occurs during animal transportation, has been reported to impair muscle glycogen repletion in cattle (McVeigh and Tarrant, 1982). Consequently, the ultimate pH of beef may vary between 5.4 and 7.2. High pH_u meat (>5.8) is usually dark coloured with a reduced flavour and is highly susceptible to bacterial spoilage (Beltran et al, 1997).

Glycolytic potential includes all the components (glycogen, glucose-6-phosphate, lactate) that can be converted into lactic acid and does not change during the post-mortem period (Monin and Sellier, 1985). Glycolytic potential is inversely related to pH_u . Przybylski et al (1994) attempted to predict the ultimate pH of bovine, porcine and ovine muscles by establishing a model based on glycolytic potential and ultimate pH. The pH_u values obtained from the study were fitted into a curvilinear regression line which showed a downward trend with increasing glycolytic potential until it reached a convergence point (Figure 2.5). They observed that after reaching this point, pH_u did not change irrespective of an increase in glycolytic potential and the convergence pH value was dependent on the animal species and muscle type. This suggests that lactic acid production and the associated pH decline stops before all the glycogen is consumed (Monin et al, 1987; Immonen and Puolanne, 2000) due to the inactivation or inaccessibility of glycogen phosphorylase.

Immonen and Puolanne (2000) demonstrated that the relationship between pH and residual glycogen concentration in beef was curvilinear. At pH values <5.8 , the residual glycogen concentration varied from 10 to 83 $\mu\text{mol/g}$. In addition, the variation in residual glycogen was independent of the ultimate pH. Therefore, other factors in addition to substrate concentration (muscle glycogen) influenced pH_u .

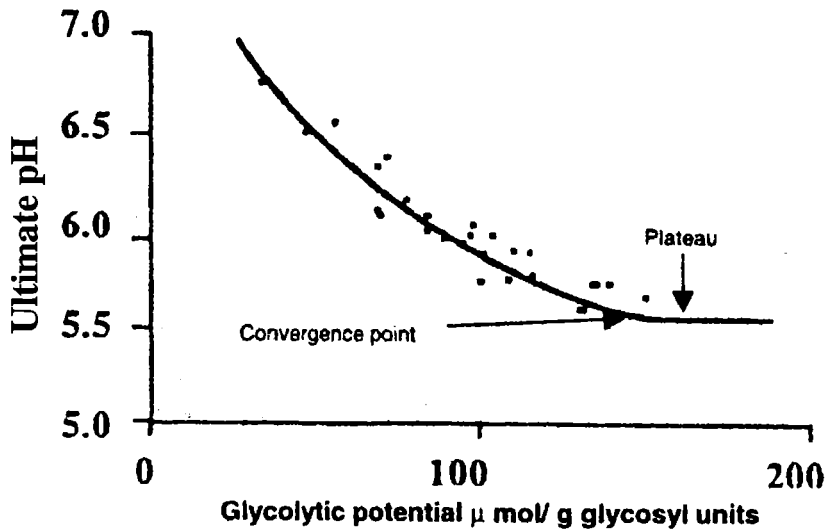


Figure 2.5: Relationship between glycolytic potential and ultimate pH of sheep pectoralis profundus (Przybylski et al., 1994).

2.3.3.2 Glycogen phosphorylase and AMP deaminase

Two enzymes may limit the glycolytic process and the resulting pH_u . They are glycogen phosphorylase and AMP deaminase (Scopes, 1974). Glycogen phosphorylase a, the active form of phosphorylase, catalyses the breakdown of glycogen to glucose – 1 – phosphate. It has been shown that high levels of glycogen phosphorylase result in a low pH_u (Scopes, 1974). Similarly, recent studies by Van Laack et al (2001) indicate that in pork muscle, glycogen phosphorylase activity explains 28% of the differences in pH_u . A high activity was associated with a lower pH_u .

Glycolysis requires glucose, ADP and phosphate (Greaser, 1986). However, as mentioned earlier, glycolysis stops if either glucose or ADP runs out. In post-mortem muscle, ADP is gradually depleted because it is converted to ATP and AMP. AMP-deaminase converts AMP to IMP (Inosine monophosphate). Therefore, if adenine nucleotides are irreversibly converted to IMP, ADP is no longer available for rephosphorylation and glycolysis stops. Van Laack (2001) found that a higher amount of AMP-deaminase was associated with a higher pH_u in beef muscle. The author also demonstrated that variation in AMP-deaminase could explain only 10% of the variations in pH_u in pork muscles. The observed differences may be because of the species and muscle-type effects on AMP-deaminase.

2.3.3.3 Muscle type and ATPase activity

The type of muscle is an important determinant of ultimate pH. White muscles, for example turkey breast muscles, have fast intermittent and mainly anaerobic action whereas red muscles, for example, turkey thigh muscle, are adapted for slower more continuous action and depend on a supply of oxygen (Swan, 1993). Ultimate pH is positively related to oxidative capacity and negatively to glycolytic capacity. The study by Hunt and Hedrick (1977) showed that cattle muscles rich in red fibres tended to have the highest ultimate pH values. The authors also reported significant differences between muscle types in beef. The differences may be attributed to the differences in function of the muscles.

White muscles have a higher ATPase activity, a larger store of glycogen to sustain anaerobic glycolysis and a lower pH_u than red muscles (Lawrie, 1992). ATPase converts ATP into ADP, Pi (phosphate) and energy. A higher ATPase activity, as in white muscles, results in a more rapid depletion of ATP and an earlier onset of rigor. However, Scopes (1974) found no direct effect of ATPase activity at ultimate pH_u .

2.4 Meat tenderness

Meat tenderness is one of the most important texture attributes through which consumers judge the quality of meat, especially eating quality (Hopkins and Fogarty, 1998). Tenderness is measured by the related physical property of the resistance to shear or the hardness of the meat. Meat with low shear force values is tender. The rate and extent of post-mortem meat tenderisation is dependent on intrinsic (species, animal age, type of muscle and muscle location) and extrinsic (pre-slaughter stress, slaughter conditions, post-slaughter handling, pH and temperature) factors. For instance, chicken breast muscle may achieve optimum tenderness within 24 hours (Lyon et al, 1992) whilst it may take up to 21 days in beef muscles.

Meat tenderness has been generally resolved into at least two different components known as 'background toughness' and 'myofibrillar toughness' (Ouali, 1991). Whereas 'background toughness' is related to the presence of collagen, which is a major constituent of connective tissues, 'myofibrillar toughness' is attributed to myofibrillar structures including the sarcomere and cytoskeletal framework (see section 2.2). During post-mortem

ageing of meat, the mechanical properties of collagen remain unaltered and, as a consequence, the improvement in meat tenderness may be attributed to the structural and biochemical alterations occurring at the level of myofibrils. It is well known that tenderness improves during post-mortem ageing of meat (Young et al, 1980; Greaser, 1986; Koohmaraie, 1988; Taylor et al, 1995). However, the exact mechanism responsible for post-mortem meat tenderisation is still unknown. Some of the proposed mechanisms are discussed below.

2.4.1 Role of sarcomere length in meat tenderisation

The shortening of sarcomere lengths has been shown to increase the initial toughness of unaged meat (Davey et al, 1967; Herring et al, 1965). The studies of Marsh and Leet (1966) revealed that beef muscles having sarcomere lengths of 2.0 to 2.5 μm were tender, those with sarcomere lengths of 1.7 to 2.0 μm were moderately tough, and those with sarcomere lengths of 1.5 to 1.7 μm were extremely tough. The authors also observed that sarcomere lengths less than 1.5 μm resulted in increasing tenderness.

Other studies have found there is little or no relationship between sarcomere length and the toughness of beef (Aberle et al, 1981; DeVol et al, 1988). Smulders et al (1990) demonstrated that tenderness was completely independent of sarcomere length in rapidly glycolyzing post-mortem beef muscles which reached pH of 6.3 or less within three hours post-mortem and that some muscles which had sarcomere lengths of 1.6 to 1.7 μm produced the most tender meat. This result suggests that there is no direct causal link between short sarcomere lengths and decreased tenderness. The wide variability in the results from different studies suggests that sarcomere length is related to some factors which, in turn, are directly associated with meat tenderisation.

Although most reports considered only sarcomere shortening, the rate of meat tenderisation between species has been related to variations in sarcomere lengthening by Takahashi (1992; 1996). The author suggests that rigor-shortened sarcomeres and the relaxation of contracted sarcomeres post-rigor largely determine the rate of meat tenderisation during ageing. This suggestion is questionable because the tenderness of muscles which were

prevented from shortening by stretching still improved during post-mortem ageing (Goll et al, 1991).

2.4.2 Mechanisms involved in meat tenderisation

During post-mortem storage, several changes occur in skeletal muscle which results in the loss of its structural integrity (Figure 2.6). Almost all researchers believe that the loss of structural integrity is responsible for meat tenderisation. However, there is debate about the mechanism of meat tenderisation. The strongest evidence suggests these changes are due to the proteolysis of myofibrillar proteins (Koohmaraie et al, 1987; Koohmaraie, 1996; Geesink and Koohmaraie, 1999a). But other researchers believe that it is due to changes in the actin-myosin interaction (Goll et al, 1991), a non-enzymatic effect of calcium (Takahashi, 1992) or a rise in ionic strength (Ouali, 1992). The major changes in skeletal muscle proteins under post-mortem storage are summarised below:

- (a) Degradation of titin filaments which connect myosin filaments in the direction of the muscle fibres from the M-line to the Z-disk (Huff-Lonergan et al., 1996).
- (b) Degradation of desmin (Young et al, 1980). These proteins are involved in the cross-linking of myofibrils and their disruption could lead to the observed post-mortem fragmentation of myofibrils.
- (c) Degradation of the myofibrillar protein, nebulin (Anderson and Parrish, 1989).
- (d) Disappearance of troponin-T and the concomitant appearance of 28-32 kDa polypeptides (Koohmaraie, 1996).
- (e) Degradation of Z-disk myofibrillar proteins which leads to the fragmentation of myofibrils (Taylor et al, 1995).

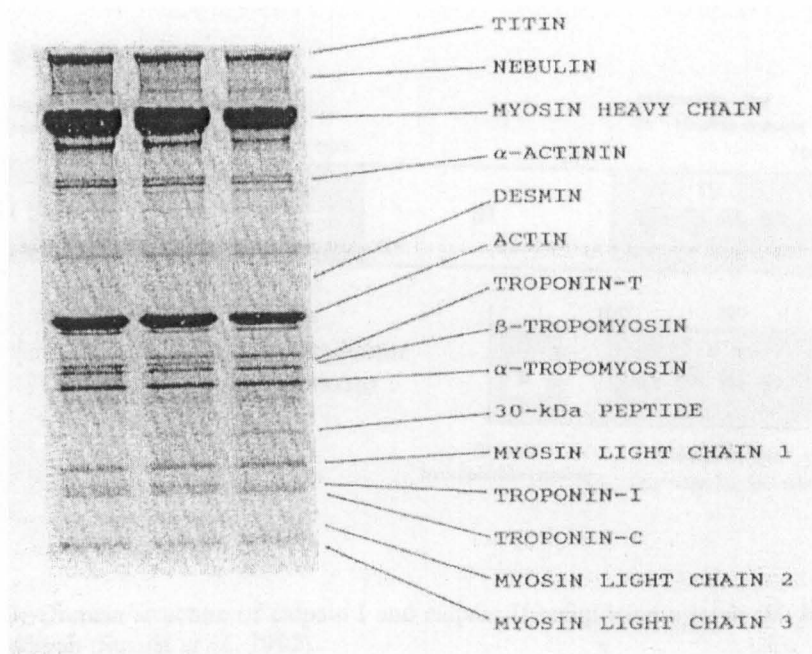


Figure 2.6: *SDS-PAGE analysis of myofibrillar proteins of lamb M longissimus muscle after 1, 7 and 14 days post-mortem storage at 2-4 °C (Geesink, 1993).*

2.4.2.1 Role of physicochemical mechanism in meat tenderisation

Various physicochemical factors have been suggested to affect the extent and rate of meat tenderisation. These include ultimate pH and the rate of pH fall in post-mortem muscles and the changes in osmotic pressure during the post-mortem storage of meat. The effects of osmotic pressure changes on post-mortem muscle tenderisation have received some attention (Ouali, 1992). It has been suggested that osmotic pressure may contribute directly to the fragilization of myofibrillar structures during meat tenderisation (Winger and Pope, 1980). According to Ouali (1990), the ionic strengths reached in post-rigor beef muscles range between 0.2 and 0.35. It has been demonstrated that these ionic strengths can create osmotic pressures sufficient to dissociate contractile proteins and, as a result, may alter the integrity of myofibrils as observed during post-mortem conditioning. Furthermore, the ionic strength of post-mortem muscle may also facilitate the hydrolytic action of endogenous proteinases. In other words, there could be a synergistic relationship between ionic strength and proteinases in the tenderising process (Wu and Smith, 1987). However, the data of Kendall et al (1993) does not support the hypothesis that the myofibrillar proteins were

solubilized in the presence of high ionic strength as a result of the osmotic pressure created in post-mortem muscles.

2.4.2.2 The role of proteolysis in meat tenderisation

As mentioned above, most of the changes in skeletal muscle which lead to the disruption of the muscle cell are due to proteolytic activities of Ca^{2+} -dependent proteases (Koohmaraie et al, 1987; Ouali, 1990). In support, Koohmaraie (1988) demonstrated that incubating muscles with calcium chloride induced proteolysis of the myofibrillar proteins and the fragmentation of myofibrils whereas incubation of muscles with calcium chelators (i.e. EDTA and EGTA) prevented myofibrillar protein degradation and myofibril fragmentation. They also showed that infusion of the carcasses with calcium chloride accelerated the degradation of myofibrillar proteins, myofibril fragmentation and meat tenderisation. These observations are insufficient however to prove that proteolysis is the cause of tenderisation, since calcium chloride could result in a direct non-enzymatic effect and a weakening of the associated myofibrillar proteins (Takahashi, 1999).

Perhaps the most important observation that points to proteolysis as the most likely cause of meat tenderisation is the report by Koohmaraie (1990) which showed that when carcasses were infused with zinc chloride, a calpain inhibitor, the degradation of myofibrillar proteins, myofibril fragmentation and tenderisation were inhibited. If the direct non-enzymatic effect of Ca^{2+} on myofibrillar proteins is the cause of meat tenderisation (Takahashi, 1999), the infusion of zinc chloride should not inhibit post-mortem meat tenderisation.

Although it is widely accepted that proteolysis of key myofibrillar proteins is responsible for meat tenderisation (Huff-Lonergan et al, 1996; Uytterhaegen et al, 1994; Taylor et al, 1995; Geesink and Koohmaraie, 1999a), the specific proteases responsible for the proteolysis of these myofibrillar proteins have been a subject for debate. Koohmaraie (1992) suggested that for proteinases to be possible candidate enzymes, they should have the following characteristics: (a) be located within the skeletal muscle cell, (b) have access to the substrate myofibrils and (c) have the ability to degrade the same proteins that are degraded during post-mortem storage. The proteolytic systems that have the potential to be

involved in post-mortem proteolysis includes: (1) the multicatalytic proteinase complex (MCP); (2) lysosomal cathepsins; and (3) the calpains. Koohmaraie (1994) reported that the maximum proteolytic activity of MCP was at pH 7.5-8.0 and, although MCP can degrade troponin-C and myosin light chain-1 and -2, the myofibrils are poor substrate for the MCP. Since MCP cannot degrade the proteins which are normally degraded during post-mortem ageing and because MCP is not very active at pH values which are usually encountered in post-mortem meat (< 5.8), MCP is unlikely to have a direct role in proteolysis responsible for meat tenderisation.

It was also thought that lysosomal proteases could play a role in proteolysis particularly at low pH when the calpain enzyme system is not very active (Harper 1999; LaCourt et al 1986). Cathepsins B, D, H, and L within the lysosomes are proteolytic enzymes and have a pH optima below 6.0. Of these, cathepsin H cannot degrade myofibrillar proteins although cathepsin D can degrade proteins below pH 5.0. However, its action in post-mortem meat tenderisation at an ultimate pH of 5.5 is minimal (Ouali et al, 1987). In contrast, cathepsins B and L are active. Cathepsin L has been shown to degrade troponins T and I, and C-protein rapidly, and titin, nebulin, α -actinin, tropomyosin, myosin and actin slowly. Because of the location of cathepsins B, D and L in the muscle cell, they would have to be released from the lysosomes in order to access the myofibrils. LaCourt et al (1986) has demonstrated that under post-mortem conditions, muscle lysosomes are not ruptured even after 28 days of post-mortem storage. In contrast, O' Halloran et al (1997) has shown that lysosomal enzymes are released into the cytosol and could contribute to post-mortem meat tenderisation.

There is evidence suggesting that the calpain proteolytic system is the underlying mechanism responsible for post-mortem proteolysis and meat tenderisation (Goll et al, 1998; Koohmaraie, 1996; Taylor et al, 1995; Ouali, 1990; Geesink et al, 2000). Some of the evidence is summarised below: (1) Calpain I and II have absolute requirement for calcium ions; elevated calcium ion concentration is responsible for the weakening of myofibrillar structures that results in tenderisation (Takahashi, 1999; Koohmaraie, 1992); (2) calcium not only does not activate cathepsins' activity, but at 10 mM inhibits cathepsin B activity by 39% (Barrett, 1973); (3) calcium has no effect on the activity of MCP (Koohmaraie, 1992); (4) of the potential proteolytic candidates, only the calpains can reproduce post-

mortem changes in myofibrils under *in vitro* conditions; (5) calcium chloride acceleration of post-mortem tenderisation is inhibited by N-Acetyl-leu-leu-norleucinal, a substrate-like inhibitor of calpains (Alarcon-Rajo and Dransfield, 1989). The inhibitor does not inhibit cathepsin B and L.

Despite the evidence that calpain has a key role in meat tenderisation, the questions still remain as to how calpain can be active in the presence of its endogenous inhibitor, calpastatin, and under pH < 6.2 and 4°C or 5°C, which are normal post-mortem conditions. Theoretically, one molecule of calpastatin can inhibit four molecules of calpain I. The activity of calpastatin in post-mortem muscle, measured *in vitro*, exceeds that of calpain I. Dransfield (1992) suggested that calpastatin is unable to inhibit the calpains at post-mortem muscle pH levels. However, Geesink and Koohmaraie (1999a) found no significant effect of pH on the inhibition of calpain by calpastatin. Other research found that calpastatin was degraded by calpain I even when calpastatin is present in excess (Doumit and Koohmaraie, 1999). Calpastatin may, therefore, be unable to inhibit calpain I completely.

In a previous study, Koohmaraie et al (1986) incubated myofibrils with calpain I at a temperature of 5°C and pH 5.5 to 5.8. They observed that calpain I retained 24 to 28% of its activity. In a similar experiment, Huff-Lonergann et al (1996) observed that proteolysis occurred when myofibrils were incubated in the presence of calpain I at pH 5.6 and 4°C. It was concluded that the observed proteolysis was similar to that which occurred in post-mortem muscle. However, Takahashi (1996) failed to reproduce the observations and did not detect any calpain I activity on myofibrils below 15°C at pH 5.7 to 5.9. Recently, work by Geesink and Koohmaraie (1999a) has shown that even in the presence of excess calpastatin, calpain I degrades myofibrillar proteins under post-mortem conditions (pH 5.8 and 5°C).

2.4.2.3 Structure and properties of the calpains

The calpains are a family of intracellular cysteine-proteinases. Two isozymes of the calpain family, calpain I and II, are ubiquitously expressed. However, some members of the calpain family, such as skeletal-muscle-specific p94 or calpain 3 and stomach-specific nCL-2, have been found to be predominantly expressed in a limited number of organs (Sorimachi et al,

1997). The calpain system also includes the calpains endogenous inhibitor, calpastatin. Of the calpains, calpain I and II have been well studied. Calpains I and II are calcium-dependent proteases and require different concentrations of calcium ions for activity. Calpain I or μ -calpain requires micromolar Ca^{2+} concentrations and calpain II or m-calpain, millimolar Ca^{2+} concentration (Goll et al., 1998).

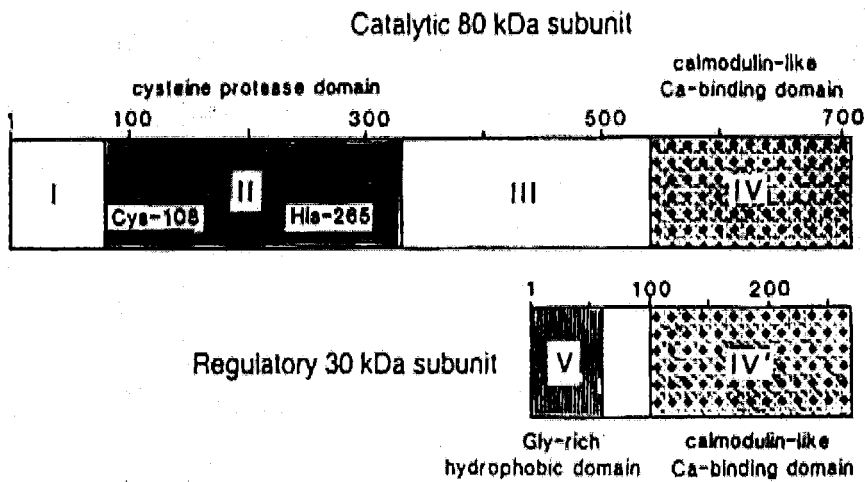


Figure 2.7: Molecular organization of calpain from chicken muscle (Suzuki, 1987).

Calpains I and II consist of a distinct large 80 kDa subunit and a common small 30 kDa subunit, forming a hetero-dimer structure (Dayton et al, 1976; Sorimachi et al, 2000). The 80 kDa contains the active or catalytic proteolytic site and the 30 kDa subunit the regulatory site (Figure 2.8). There are four domains in the large subunit (Imajoh et al, 1988). It seems domain I promotes the conversion of the inactive proenzyme (pro-calpain) to active enzyme (calpain). Domain II contains the active proteolytic site of calpain. Whilst domain III is involved in the binding of calpastatin to calpain, domain IV is responsible for binding Ca^{2+} to the proteinases (Melloni and Pontremoli, 1991).

The specific calpain inhibitor protein, calpastatin, is also expressed ubiquitously in animal cells and regulates calpain activity. Calpastatin is a monomer with a molecular mass of 105-110 kDa (Goll et al, 1998). Calpastatin has an N-terminal region called domain L followed by four other domains. Each of these four domains is able to interact with calpain (Imajoh et al, 1987). Calpain can degrade calpastatin and produce four fragments, which still retain inhibitory activity (Nakamura et al, 1989). Hence, in theory, one molecule of

calpastatin should inhibit four molecules of calpain. However, this potential inhibitory capacity of calpastatin may not be expressed *in vivo* (Pontremoli et al, 1986).

It is important to note that the mechanism of inhibiting calpain by calpastatin is competitive and fully reversible leading to fully active calpain and calpastatin after dissociation of the enzyme-inhibitor complex (Pontremoli et al, 1986). Calpastatin can be present in different isoforms in cells (Lee et al, 1992). The physiological function of these isoforms is unclear. It may be that the isoforms differ in their ability to inhibit calpain activity. The serine residues of the inhibitor can be phosphorylated (Adachi et al, 1991), which alters the ability of calpastatin to inhibit the proteolytic activity of calpain I or II (Salamino et al, 1994).

Cytoskeletal proteins have been identified as the preferred substrates of the calpains in all animal cells (Croall et al, 1986; Shoeman and Traub, 1990). The ability of calpain to degrade myofibrillar proteins is, however, limited (Goll et al, 1992). For example, the major muscle protein, myosin, is very slowly degraded by calpain I and undenatured actin is not cleaved at all. In contrast, calpain I and calpain II rapidly cleave troponin T, desmin, vinculin, talin, spectrin, nebulin and titin, and more slowly troponin I, filamin, C-protein, dystrophin, and tropomyosin. α -actinin and M-protein are cleaved very slowly (Goll et al, 1998). The incubation of calpains with myofibrils, results in the loss of the Z-disks which leaves a gap in the middle of the sarcomere. Since α -actinin is a major component of the Z-disk, the loss of Z-disk structure could be due to the release of α -actinin from the Z-disks in a nearly intact form (Goll et al, 1991).

Calpains can cleave several cytoplasmic (sarcoplasmic) proteins including most protein kinases and phosphatases. It has also been reported that calpains can degrade receptor proteins such as hormones (Vedeckis et al, 1980) and growth factor receptors (Ek and Holdin, 1986). However, calpains do not cause the bulk degradation of sarcoplasmic proteins to small fragments (Tan et al, 1988).

One of the characteristics of calpain I and II is that the binding of Ca^{2+} induces autolysis in a series of defined steps with eventual loss of activity (Koohmaraie, 1996; Suzuki et al, 1981). The extent of autolysis is a measure of calpain activity under post-mortem conditions; high autolysis indicates increased calpain activity. During the post-mortem

period, calpain I gradually loses its activity whilst the activity of calpain II remains relatively constant (Vidalenc et al, 1983; Ducastaing et al, 1985; Morton et al, 1999). Consequently, it was suggested that only calpain I is activated during the post-mortem period and, therefore, calpain I rather than calpain II may be responsible for post-mortem meat tenderisation. It was initially thought that the loss of calpain I activity is a consequence of its extensive autolysis (Koohmaraie et al, 1987). However, Geesink and Koohmaraie (1999b) showed that the initial autolysis steps reduce the 80 kDa subunit of calpain I to 76 kDa through a 78 kDa intermediate and there was no loss of activity (Figure 2.9). The loss of calpain I activity during post-mortem storage is, therefore, not a result of extensive autolysis but due to the instability of the autolyzed calpain I at low pH and high ionic strength (Geesink et al, 2000).

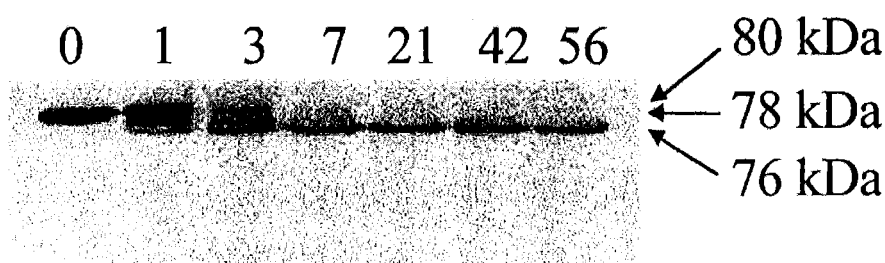


Figure 2.8: *Western blot analysis of the large subunit of calpain I in the soluble fraction of lamb during 56 days of ageing at 4°C (Geesink and Koohmaraie, 1999b). The arrows indicate the position of the native 80 kDa and partly autolyzed 78 and 76 kDa fragments.*

2.4.3 Relationship between pH, tenderness, MFI and sarcomere length

The relationship between ultimate pH and tenderness has been demonstrated for beef (Purchas, 1990; Purchas and Aungsupakorn, 1993), sheep (Watanabe et al, 1996; Devine et al, 1993; Bouton et al, 1971) and pork (Dransfield et al, 1994). However, its nature remains controversial. Guignot et al (1994) and Bouton et al (1973) found a linear relationship between ultimate pH and meat tenderness, whereas other researchers (Jeremiah et al, 1991; Watanabe et al, 1996; Purchas, 1990) found a curvilinear relationship with minimum tenderness occurring between pH 5.8 and 6.2 (Figure 2.7). Meat with an ultimate pH below or above the 5.8-6.2 range was more tender. The reason for this phenomenon is not yet clear.

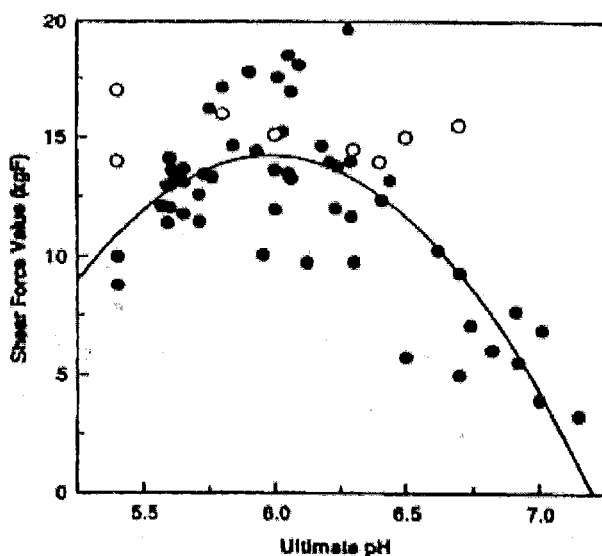


Figure 2.9: Post-mortem changes in shear force of sheep loin muscles treated with $ZnCl_2$ (o) and without $ZnCl_2$ (•) after 1 day of ageing (Watanabe et al, 1996).

There have been some suggestions to explain the curvilinear relationship. Yu and Lee (1986) suggested that there is less proteolytic activity at the intermediate ultimate pH values (5.8-6.3) because the pH optimal of the two separate enzyme systems is outside the range. Thus, in high ultimate pH meat (>6.3), the activity of calpains is favoured (activity maximal at neutral pH) and at low pH (<5.8), the activity of the cathepsin is enhanced. This suggestion may not offer a satisfactory explanation for the curvilinear relationship between ultimate pH and meat tenderness because it has been shown in numerous studies that the cathepsins play no major role in post-mortem meat tenderisation (Ouali et al, 1987; Koohmaraie, 1988).

Proteolysis due to calpains results in the weakening/fragmentation of myofibrillar proteins. Myofibrillar fragmentation index (MFI) is a measure of proteolysis and provides a measure of the tenderisation process. It measures the extent of ageing through changes in the fragility of the myofibrils which proceeds through the weakening of the Z-line proteins. As a result, when homogenised, the myofibrils break into increasingly small fragments as the ageing process progresses. Watanabe et al (1996) studied the relationship between MFI and ultimate pH of lamb LD muscles. These authors observed that at one day post-mortem, the MFI was slightly higher at pH <5.8 and >6.3 compared to the pH range 5.8-6.3. This

implies that the toughness of intermediate pH meat may be due to the slow rate of ageing of the muscles. However, other researchers have reported that the increased toughness of beef of intermediate pH is not necessarily due to reduced myofibrillar protein degradation even after 20 days of ageing (Purchas and Yan, 1997). The inconsistencies between these two reports may suggest that mechanism(s) other than proteolysis may be involved in the toughness of intermediate pH meat.

Non-enzymatic causes have also been suggested to explain the curvilinear relationship between ultimate pH and meat tenderness. Purchas and Aungsupakorn (1993) and Purchas (1990) have suggested that the decrease in sarcomere length as the pH increases to 6.2 may, at least in part, explain the higher level of toughness of intermediate pH.

2.5 Poultry meat quality

Poultry meat production has increased rapidly with an increased output of 43% between 1995 and 2000 compared to 29% and 9% increases in beef and pork meat production, respectively (Berri, 2000). This increase has been in response to increased demand for chicken and turkey portions, and their processed products. Poultry products have been marketed on health benefits and consumers' perception of consistent tenderness. Wakefield et al (1989) demonstrated that about 15% of chicken carcasses produce tough breast meat after cooking. There are anecdotal reports in New Zealand that consumers are concerned about the inconsistency in turkey breast meat quality. Consequently, it will be worthwhile to investigate the causes and possible solutions which will help the industry to optimise its processing techniques. This will allow the poultry industry to produce consistently tender meat and increase the demand for poultry meat.

Inconsistency in poultry meat quality may result from several intrinsic and extrinsic factors. Intrinsic factors such as age and sex have been reported to influence chicken meat texture. Mohan et al (1987) reported that at six weeks of age, male chickens produced tougher meat than females. However, at eight weeks of age the meat from females was tougher. Variation in turkey and chicken meat quality can also be influenced by extrinsic factors such as pre-slaughter stress and processing conditions. The processing conditions which have been shown to influence the textural characteristics of poultry meat quality include: method of stunning, scalding, plucking, chilling and freezing carcasses, and deboning time (Berri,

2000). These factors exert their influence through post-mortem biochemical changes such as onset of rigor, rate and extent of post-mortem glycolysis (section 2.3). Although most of the research work is on beef and pork meat, the biochemical changes in post-mortem turkey or chicken meat, which influence meat quality are similar. The impact of some of these factors on turkey and chicken meat quality are discussed below.

2.5.1 Effect of pre-mortem and post-mortem conditions on poultry meat quality

2.5.1.1 Pre-slaughter stress

Turkeys and chickens are exposed to a number of stressors prior to slaughter. These stressors include feed withdrawal, harvesting, crating, transport, handling operations and temperature variations (Sams and Mills, 1993). Many of these stressors cause injuries such as broken bones, scratches, bruising and haemorrhages and increase the incidence of carcass downgrading (Barbut et al, 1990). More subtly, stressors may affect the quality of turkey and chicken meat by altering the physiology and metabolism of muscles. The effect of stressors on muscle metabolism depends on the type and intensity of the stressors.

In turkey and chicken processing plants, it is a common practice to withdraw feed from the birds prior to slaughter. Feed withdrawal depletes the glycogen content of muscles, which results in an increase in the ultimate pH of the muscle (Sams and Mills, 1993). As a result, the potential for rigor shortening is reduced and consequently, broiler breast meat tenderness is improved (Lyon et al, 1991; Sams and Mills, 1993). However, although the meat is more tender, meat colour is darker (Chen et al, 1991).

Responses to stress that have been observed in broiler breast meat as a result of transportation (Kannan et al, 1997) include reduced tenderness, lower water-holding capacity (Ehinger, 1977) and pale colour (Cashman, 1987). Heat stress prior to slaughter has been reported to increase the rate and extent of pH fall in chicken muscles (Holm and Fletcher, 1997) and to produce tough meat. Similarly, turkey muscles exposed to pre-slaughter stressors such as cold, heat or struggling, exhibit a rapid fall in pH, which may, eventually, lead to PSE-like, and tough breast meat (Sosnicki, 1993).

2.5.1.2 Stunning

The main reason for stunning turkeys is to immobilise the birds to allow an accurate neck cut and a uniform rate of bleeding which will ensure death before scalding. Stunning also lowers the incidence of carcass and meat downgrading (Berri, 2000). In turkey processing plants, stunning is achieved by the application of an electric current. The electrical stunning parameters vary from country to country. In Europe, minimum currents of 125 mA and 250 mA have been recommended for chickens and turkeys, respectively (Gregory and Wotton, 1991). In this experiment, a current of 160 mA was used. Apart from electrical stunning, gas stunning may be used in poultry processing plants in countries like the UK (Berri, 2000).

Research has shown that stunning changes muscle metabolism by reducing struggling during killing (Papinaho et al, 1995). Consequently, stunning delays the onset of rigor mortis by slowing the ATP and pH decline without altering the ultimate pH (Papinaho and Fletcher, 1996; Northcutt et al, 1998a; Murphy et al, 1988). The slower metabolism reduces the risk of rigor or heat shortening, which occurs when both the muscle temperature and glycolysis rate are high (Dunn et al, 1993b). Studies by Raj et al (1990) have also shown that meat from stunned birds was more tender than those from non-stunned carcasses.

However, because stunning slows the rate of post-mortem glycolysis, it may favour 'cold shortening' (see section 2.3.2), resulting in tough meat, particularly from early harvested and rapidly chilled breast muscles. This phenomenon has been observed in poultry when muscle temperature is very low ($<5^{\circ}\text{C}$) and the pH is still high (Dunn et al, 1993a). Furthermore, electrical stunning may reduce the overall quality of turkey and chicken breast meat if the appropriate current parameters are not applied. For example, increasing current intensity or decreasing frequency produces a higher incidence of muscle haemorrhages and broken bones (Gregory and Wilkins, 1990; Wilkins et al, 1999).

2.5.1.3 Chilling and Boning

The post-mortem temperature of the muscle strongly interacts with the rate of rigor mortis development in determining the quality of poultry meat (Wakefield et al, 1989; Sosnicki et

al, 1998). Slow cooling of muscles with rapid rigor may induce 'heat-shortening' and PSE-like meat by increasing protein denaturation whilst rapid cooling will toughen slow glycolysing muscles. At present, rapid chilling (immersion chilling) is being used in the processing of turkey breast meat to prevent defects (PSE-like) in meat quality. However, the rapid chilling of turkey carcasses could be a source of variation in the tenderness of the resulting breast meat.

Furthermore, the delay prior to breast harvesting is also important for meat quality. Perhaps it is largely responsible for the variations in the tenderness of broiler and turkey breast meat. In the last decade, the demand for cut and further processed turkey and broiler products has increased dramatically. In order for processors to meet this demand, under the constraints of an efficient plant operation including requirements for reduced labour, handling and storage, it has become common practice to remove meat from carcasses immediately after chilling (Owens and Sams, 1997; Thompson et al, 1987). However, muscles removed from bones prior to rigor completion will use any ATP produced by the glycolytic pathway to contract, causing shortened muscles and tough meat (Lyon et al, 1985; Stewart et al, 1984). In chickens, both Lyon et al (1985) and Dawson et al (1986) found that variations in toughness were associated with the time of boning. Thus, processors deboning turkey breast muscle at three hours post-mortem, risk producing meat which is unacceptably tough to consumers.

Chapter 3

Materials and Methods

3.1 Materials

Potassium phosphate (mono and dibasic), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium potassium tartrate, KCl, EDTA, NaN_3 , Tris, NaCl and NaOH were obtained from BDH Inc. Bovine serum albumin (BSA), EGTA, Tween 20, anti-troponin T (clone JLT-12), anti-desmin (D8281), secondary antibody conjugated to alkaline phosphatase (goat anti-mouse) were obtained from Sigma. Acrylamide/*N,N'*-bis-(methylene acrylamide) solution, sodium dodecyl sulphate (SDS), nitrocellulose, 2-mercaptoethanol (MCE), glycerol, bromophenol blue, Coomassie brilliant blue R-250, glycine were obtained from Bio-Rad.

3.2 Temperature and pH measurements

The temperature and pH of turkey breast muscle was measured on the processing line at various post-slaughter time points. A single incision was made in middle of the breast muscle. The pH and temperature of the breast muscle was measured by inserting into the cut an Orion 8163 glass electrode pH and temperature probes attached to a Hanna HI 9025 portable pH meter. At Lincoln University, the one day and seven days post-mortem temperature and pH measurements were determined by directly inserting the pH and temperature probes into the main muscles and the fillets.

The beef muscle pH at 24 hours and seven days post-mortem was determined by cutting 2 g from each of the meat samples and homogenising this for 30 seconds in 30 ml of deionised water. The pH of the homogenate was measured using Hanna (HI9025) with an Orion Ross probe (8163S).

3.3 Tenderness and cooking loss determination

Tenderness and cooking loss meat samples were stored at -20°C until analysed. The shear force of both beef and turkey samples was determined using a MIRINZ tenderometer

(Chrystall and Devine, 1991). At Lincoln University, the frozen meat samples were thawed overnight at 2°C before cooking (Belk et al., 2001). The samples were cooked individually in plastic bags immersed in a water bath maintained at 80°C until the samples reached an internal temperature of 75°C, as measured using Fluke Type K temperature probes attached to Fluke 52 meters. The cooked meat samples were immediately cooled on ice for about 20 minutes (Bickerstaffe et al., 2001). After cooling, 8-10 pieces (10 x 10 mm square by 25 mm long) were removed from the samples parallel to the muscle fibre directions. Each of the pieces was cut individually using the tenderometer. The mean reading of the tenderometer (Pa) was converted to shear force (kgF) using the regression equation: $\text{KgF} = (\text{kPa} \times 0.2) - 1.7$

The cooking loss was determined for the turkey breast muscles at 24 hours and seven days post-mortem. The breast meat samples were wiped clean with a paper towel before cooking and weighing. After cooking and cooling in ice, the meat samples were dried gently with a paper towel and reweighed. The differences between the weights of the samples before and after cooking were calculated as the percentage cooking loss.

3.4 Biochemical assays

3.4.1 Myofibril isolation

This procedure was carried out on both 24 hours and seven days of aged beef and turkey samples according to Culler et al (1978) with modifications. Myofibrils were isolated from the meat samples by homogenising 2 g of muscle tissue using a Polytron blender on high (30 seconds for beef muscle and 10 seconds for turkey breast muscle) in 20 ml of cold isolating medium containing 100 mM KCl, 1 mM EDTA and 1 mM sodium azide in 20 mM potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 1000xg for 15 minutes at 2°C. A sample (500 µl) of the supernatant was solubilized in 100 µl of 6xSDS-PAGE buffer [0.35 mM Tris/HCl (pH 6.8), 10.28% SDS, 30% glycerol, 5% MCE and 0.01% bromophenol blue], for further analysis while the remaining supernatant was discarded. The pellet (sediment) was resuspended in 20 ml of the original isolating medium buffer, centrifuged again at 1000xg for 15 minutes and the supernatant decanted. The sediment was again resuspended in 10 ml of the MFI buffer, vortexed and passed through a

polyethylene strainer to remove connective tissues and debris. Filtrate (500 µl) was solubilized in 6xSDS-PAGE buffer which was used to analyse the extent of degradation of myofibrillar proteins. The remainder was used for MFI and sarcomere length determinations.

3.4.2 Protein concentration determination

The protein concentration in the suspension was determined by the biuret procedure (Culler et al., 1978). 0.5 ml of 10% NaOH was added to 0.5 ml of the suspension in a glass tube and 4 ml of Biuret reagent [700 ml of 6 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 20 mM sodium potassium tartrate ($\text{NaKC}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) + 300 ml 10% (w/v) NaOH] was added. The tubes were incubated for 30 minutes at room temperature. Bovine serum albumin (BSA) of 0, 2, 4, 6, 8 mg/ml (1.0 ml per concentration level also dissolved in 10% NaOH) were used as standards. The samples and standards absorbance at 540 nm were read in duplicate using a Unicam 8625 UV/VIS spectrophotometer. Using the protein standard curve obtained from the BSA samples, the protein concentrations (mg/ml) of the myofibril suspensions were determined.

3.4.3 Myofibril fragmentation index (MFI) determination

The MFI was determined according to Geesink et al (2001). An aliquot of the myofibril suspension from above was diluted with the MFI buffer to 0.5 mg/ml protein concentration. After obtaining the required protein concentration, the diluted myofibril suspension was measured at 540 nm. The MFI values were recorded as absorbance units per 0.5 mg/ml myofibril protein concentration multiplied by 200.

3.4.4 Sarcomere length determination

The sarcomere length was determined on myofibril suspensions prepared for MFI measurements. The sarcomere lengths of at least 20 myofibrils per sample were determined. Each sample preparation (7.5 µl) was placed on a slide and covered with a cover slip. The preparation was examined with a Zeiss Photomicroscope equipped with phase contrast and polarised light optics. Photographs of the myofibrils at a magnification

of 1000x were recorded on Kodak film with 400 speed (36 exposures) for the beef samples. The sarcomere length was determined by measurement on the photographs, using photograph of a micrometre as a standard. However, turkey myofibril preparations were examined with a LEICA DMLD photomicroscope equipped with phase contrast and polarised light optics. The images were analysed with SPOT RT software v 3.2 (Diagnostic Instruments, Inc., USA.) and the sarcomere lengths were determined using the software, Image-Pro plus version 4.5 (Media Cybernetics: The Image Experts, USA.).

3.4.5 SDS-PAGE and immunoblotting

The protein concentration was determined using the procedure described by Karlsson et al (1994). The samples were solubilized in Laemmli buffer [62.5 mM Tris-HCl (pH 6.8), 2.35% SDS, 1 % MCE, 10% glycerol, 0.001% bromophenol blue]. The solubilized samples were heated at 96°C for 5 mins and centrifuged in a Microfuge (10,000 g) for five minutes. Then, the SDS-solubilized material was diluted with the Laemmli buffer by a factor of 20. 100 µl was applied to a microtiter plate and 100 µl of 60% TCA was added to each of the samples. Next, the turbidity of the preparation along with that of BSA standards (0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was measured at 570 nm after 30 minutes in a microplate reader (Falcon 3072 96). Using the BSA protein concentration standard curve, the protein concentrations for the samples were determined and the original samples were diluted accordingly to a fixed concentration of 2 mg/ml.

Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) was done according to Geesink et al. (2000) using 0.75 mm thick 12.5% (37.5:1 ratio of acrylamide to N,N-methylene-bis acrylamide) separating gels. SDS-PAGE was performed in a running buffer (25 mM Tris, 0.19 M glycine, 3 mM SDS). The proteins from SDS-PAGE were transferred electrophoretically from the gels onto nitrocellulose membrane (Amersham, Buckinghamshire, UK) according to the procedure described by Towbin et al (1979). After the transfer, the membranes were first incubated in TBS (20 mM Tris, 500 mM NaCl, pH 7.5) containing 0.05% Tween-20 and 3% non-fat dried milk (NFDM) for at least 30 minutes to block non-specific binding sites. After the incubation, the TTBS/milk solution was discarded.

Next, the blots were incubated with antibody raised against Desmin (D3) and Troponin-T (CT3) in TTBS + 2% NFDM for one hour. The western blots were then washed three times with TTBS. The secondary antibody used was alkaline phosphatase conjugated anti-mouse IgG (Sigma Chemical). Antibodies were diluted in blocking buffer and incubated for one hour at room temperature with gentle shaking. The membranes were washed three times with TTBS after incubation. Then, the colour was developed by incubating the blots with BIORAD alkaline phosphate substrate BCIP/NBT (0.3 ml A + 0.3 ml B in 40 ml buffer) for 30 mins. Finally, the blots were washed extensively with water, and air dried. The western blot for the extent of autolysis of calpain I was carried out as described for desmin and troponin-T except that 7.5% separating gels were used and the antibodies were specific for calpain I. Troponin-T, desmin and calpain I were quantified from the developed dry blots by using a UVP software package.

3.5 Statistical analysis

Data was tabulated in Microsoft Excel spreadsheets, statistical analysis used Minitab Statistical Software Package version 13. Data was analysed using ANOVA, Student's t-test and regression unless otherwise stated.

Chapter 4

4.0 Effect of pre-slaughter stress on beef quality

4.1 Introduction

The management procedures imposed on the animal prior to slaughter can induce physiological and behavioural stress responses. Stress can be seen as the general expression referring to the physiological adjustments such as the changes in body temperature, heart rate, rate of respiration and blood pressure that occur during the exposure of an animal to adverse conditions. Schaefer et al (1997) defined stress as any physiological departure from normal. Stress usually leads to a reduction in meat quality (McVeigh and Tarrant, 1982).

Stress in animals may reduce the glycogen content of ante-mortem muscle. The rate and extent of glycogenolysis depends on the animal species, muscle type and the level of the stress (see section 2.3.3.1). Post-mortem muscles of stressed animals produce less lactic acid and consequently, meat of high ultimate pH. The ultimate pH in post-mortem bovine muscle may vary between 5.4 and 7.2 depending on the intensity of the stress (Beltran et al, 1997). High pH usually results in dark coloured meat with reduced flavour that is susceptible to bacterial spoilage (Lister et al, 1981). Meat showing this characteristic is perceived as being of low quality although the meat is very tender (Tornberg, 1996). The effect of ultimate pH on meat tenderness is more complex (see section 2.3.3). There is evidence that tenderness decreases with increasing pH up until the intermediate ultimate pH range of 6.0-6.2. Above this pH range, the meat is more tender (Purchas, 1990; Watanabe et al, 1996).

This experiment investigates the effect of spray washing cattle on the quality of meat. Spray washing is a hygiene requirement introduced by New Zealand Ministry of Agriculture and Fisheries (MAF) to reduce the contamination of carcasses but the process may stress the animals. The hypothesis in this research is that this stress will lead to meat with a high ultimate pH and, as a consequence, an effect on meat quality characteristics. The objectives of this chapter were to assess the effects of spray washing on the meat quality attributes of

beef, and determine whether the toughness of meat of intermediate pH is a result of sarcomere contraction or the extent of proteolysis.

4.2 Materials and methods

A herd of cattle (Devon X) was split into two groups. The first group of 10 were held in pens without any belly wash treatment (dry pens) and the second group of 11 were held in a pen where belly sprays were turned on every hour for five minutes. Animals from the dry pens were killed first whilst the spray-washed animals were killed later in the day.

The following procedures were performed on each *longissimus dorsi* (LD) samples from the two groups of carcasses: Measurement of ultimate pH, shear force determination at one day post-mortem, myofibril isolation and myofibrillar fragmentation index (MFI) determination at one day and seven days, sarcomere length measurement, and Western blotting of calpain I, troponin T and desmin (see chapter 3).

4.3 Results

4.3.1 Ultimate pH and tenderness

There was no significant difference between the ultimate pH (pH_u) of meat from spray-washed animals compared to those from the control animals (Table 4.1). However, the mean pH_u was slightly higher in the spray washed animals compared to the controls. The pH_u ranged from 5.52-6.48 and 5.50-5.79 in muscles from spray washed animals and the control animals respectively. The results indicate that there was a trend towards an increased toughness ($p = 0.089$) as a result of spray washing, although there was no significant difference between the tenderness of meat from the control animals compared to those from the spray washed animals. However, the mean tenderness of meat samples from the treated animals (8.06 kgF) was higher than from the control animals (6.30 kgF). Meat derived from spray washed animals showed higher variation in shear force values (5.2-14.5 kgF) than meat from the control animals (4.8-8.6 kgF).

The relationship between the pH_u and shear force is shown in Figure 4.1. The pH_u was positively correlated with the shear force at one day post-mortem ($p = 0.760$). From Figure

4.1, it can be seen that as the pH_u increases above 5.4 the shear force increases. However, as the pH_u increases above 6.2, the shear force tended to decrease. The result was best fitted into a curvilinear relationship.

Table 4.1: Simple statistics for the effect of the treatment on pH, shear force, MFI, sarcomere length, degradation of troponin-T and desmin, and extent of autolysis of calpain I.

Measurement	Mean \pm SE		p-value (95% C I)
	Control (n = 10)	Spray-washed (n = 11)	
pH	5.58 \pm 0.03	5.77 \pm 0.12	0.14
Shear force (kgF)	6.30 \pm 0.37	8.07 \pm 0.89	0.089
MFI (1 day)	68 \pm 4.9	71 \pm 2.7	0.67
MFI (7 days)	84 \pm 2.3	86 \pm 4.8	0.40
Sarcomere length(μ m)	1.86 \pm 0.04	1.73 \pm 0.05	0.069
Cal (B1/B2)	1.81 \pm 0.13	2.42 \pm 0.63	0.37
T (D7/D1)	0.74 \pm 0.04	0.76 \pm 0.10	0.88
De (D7/D1)	0.03 \pm 0.02	0.17 \pm 0.07	0.079

MFI = myofibrillar fragmentation index; Cal (B1/B2) = extent of calpain I autolysis; T (D7/D1) = the extent of troponin-T degradation; De (D7/D1) = extent of desmin degradation.

4.3.2 Sarcomere length and myofibrillar fragmentation index (MFI)

The extent of muscle contraction was measured as the sarcomere length. Although the treatment does not show significant effects on the sarcomere length ($p = 0.069$), the mean sarcomere length was slightly lower for muscles from spray washed animals (1.73 μ m) compared to the controls (1.86 μ m) (Table 4.1). Figure 4.1 also shows the relationship between the ultimate pH and sarcomere length. This mirrored the relationship between ultimate pH and shear force in that as the pH_u increased from around 5.5 towards the intermediate pH, the sarcomere length decreased until a minimum of 1.40 μ m which was reached at an pH_u of approximately 6.1. This curve suggests that the shorter sarcomere lengths may be the cause of the increased shear force. Correlation analysis showed that pH_u was inversely related to the sarcomere lengths ($r = -0.525$) (Table 4.2)

Table 4.2: Correlation coefficients of the parameters measured.

	pH	SF (KgF)	MFI (day1)	MFI (day7)	Sarco (µm)	Cal (B1/B2)	T (D7/D1)
SF (KgF)	0.760**						
MFI (Day 1)	0.247	-0.012					
MFI (Day 7)	0.547**	0.282	0.430				
Sarco (µm)	-0.525**	-0.512**	0.005	-0.187			
Cal (B1/B2)	-0.250	-0.019	-0.364	-0.634**	0.029		
T (D7/D1)	0.547**	0.388	0.268	0.506**	-0.517**	-0.376	
De (D7/D1)	-0.373	-0.207	-0.284	-0.570**	0.060	0.364	-0.043

** indicates correlation at $p < 0.05$, $n = 21$

There was no significant difference between the MFI (one or seven days) of meat samples from the spray wash and control animals (Table 4.1). However, the MFI of the muscles from both the control and spray-washed animals was significantly lower at day 1 compared to day seven post-mortem. At one day postmortem, it appears that low ultimate pH (5.54) or high ultimate pH (6.48) meat samples have high MFI values. However, after seven days of ageing the MFI increased linearly as the ultimate pH increased.

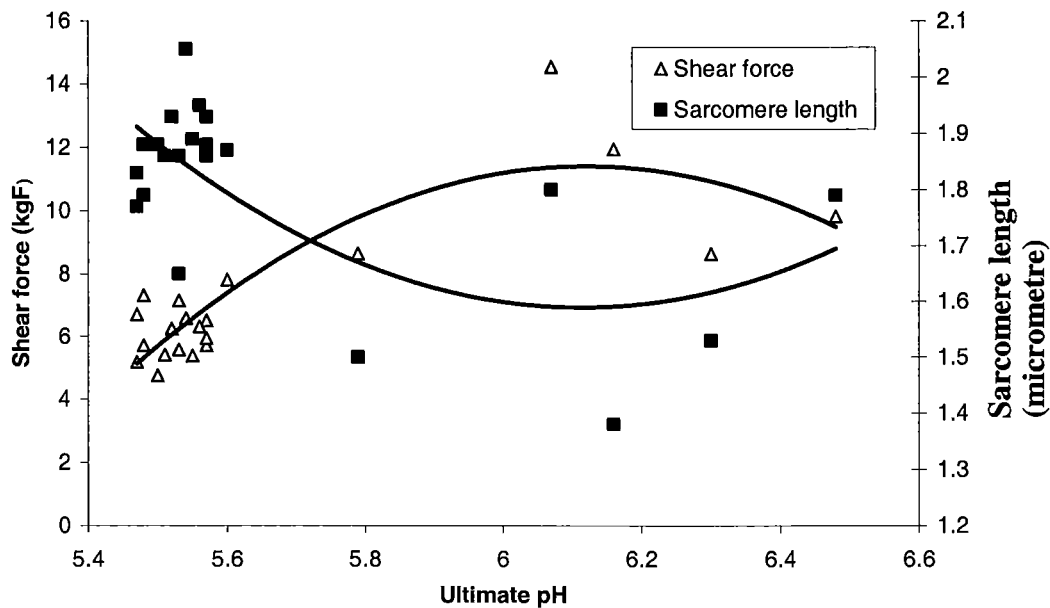


Figure 4.1: Relationship between ultimate pH, shear force and sarcomere length.

4.3.3 Autolysis of calpain I

The extent of autolysis of calpain I was determined by the ratio of the 80 kDa to 76 kDa (Figure 4.2). It was found that this ratio was inversely related to the MFI at seven days of ageing ($r = -0.517$). Because the autolysis of calpain I is a measure of calpain I activity, the increase in MFI may be due to increased proteolysis of myofibrillar proteins by calpain I. However, the extent of autolysis of calpain I was not related to the MFI at one day postmortem. Also, there was no significant difference between the autolysis of calpain I of meat from animals that were spray washed compared to the control animals.

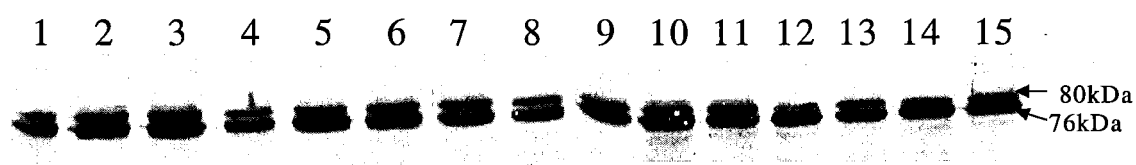


Figure 4.2: Western blotting against calpain I in the soluble fraction of the mofibril extracted at one day post-mortem. Lanes 1 to 8 were from the control animals and lanes 9 to 15 were from the spray-washed animals.

4.3.4 Degradation of desmin and troponin-T

The extent of desmin degradation (Figure 4.3), expressed as the ratio of desmin at seven days to the amount of desmin at one day postmortem, was not significantly different in the meat from animals that were spray washed compared to the control animals. However, there was a trend towards a significant difference ($p = 0.079$). The ratio of desmin at seven days to the amount of desmin at one day postmortem was inversely related to the MFI at seven days ($r = -0.570$). This indicated that desmin degradation might have contributed to the fragility of the myofibril and, as a consequence, the observed increase in MFI at seven days of ageing.

Degradation of troponin-T was not significantly influenced by the treatment at seven days of ageing, although there was increased degradation of troponin-T over the six days extended ageing period (Figure 4.3).

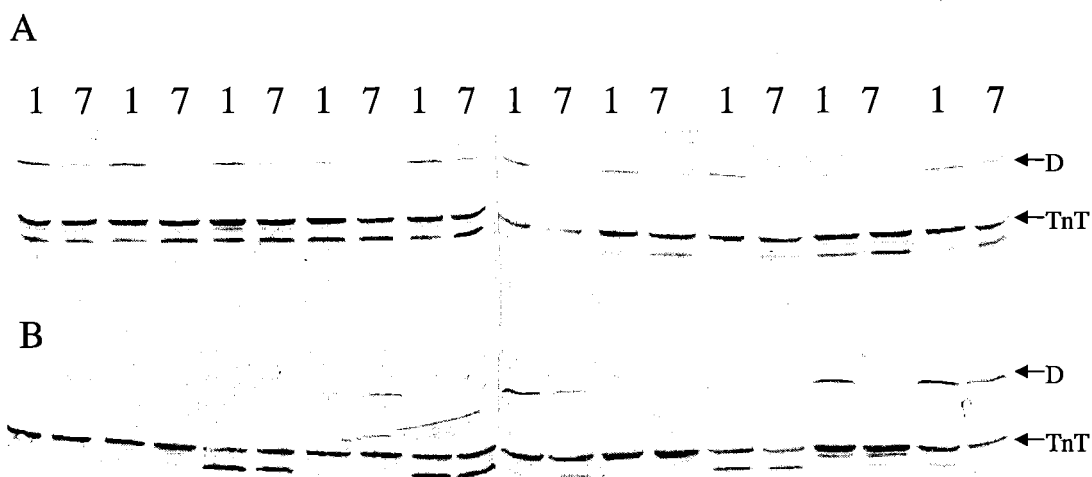


Figure 4.3: Western blot against troponin T (TnT) and desmin (D) in the myofibrillar fraction of beef samples at one day (1) and seven days (7) post mortem. A): controls (n = 10), B): spray-washed animals (n = 10).

4.4 Discussion

In post-mortem muscle, the fall in pH is due to the accumulation of lactic acid, through anaerobic glycolysis. The pH will decline until an ultimate pH (pH_u) is reached (see section 2.3.3). In this research, beef LD muscles from the spray-washed cattle were analysed for their pH_u and shear force at one day postmortem. The pH_u ranged from 5.50 to 6.48 which is consistent with a previous report on beef (Beltran et al, 1997). Spray washing resulted in considerably more variation in the pH_u of the meat (5.52 to 6.48) compared to meat from the dry animals (5.50 to 5.79). The increased variability in pH_u was reflected in the meat shear force values from the spray-washed animals which ranged from 5.2 to 14.5 kgF while that from the dry animals ranged between 4.8 and 8.6 kgF. The mean pH_u and the shear force of the meat from the spray-washed animals were higher but the difference was not significant. This may be due to the high variability of the measured parameters from the spray-washed animals.

The LD samples were also analysed to determine the cause of any toughening of the meat from the spray-washed animals. Two factors which may contribute to meat toughness are: (i) the state of contraction of the muscle (measured as sarcomere length) and (ii) the extent of any post-mortem proteolysis (measured as myofibrillar fragmentation index). The LD

from spray-washed animals had shorter sarcomere lengths than those from dry animals. This is one possible reason for the slightly increased meat toughness associated with the spray washed animals. However, the myofibrillar fragmentation index at 1 day and seven days post-mortem were essentially the same for both spray-washed and non-sprayed animals. Thus, there was no evidence that spray washing contributed to the toughening of beef LD through reduced protein degradation. Although there was no significant difference between the MFI of muscles from spray-washed and non-spray animals, the MFI was significantly higher at seven days compared to 1 day post-mortem. This result is consistent with the reports of Watanabe et al (1996), and Geesink and Koohmaraie (1999a), which showed an increased myofibrillar protein fragmentation as a consequence of proteolysis during post-mortem ageing of meat.

Post-mortem proteolysis is generally considered to be due to the action of calpain I (Koohmaraie, 1996; Geesink et al, 2000). Once calpain I is activated it not only degrades other proteins but also degrades itself, a process known as autolysis. Thus, it is possible to measure the activity of the calpains by determining the extent of calpain I autolysis. Western blotting of calpain I showed that the extent of autolysis one day postmortem was related to the MFI at seven days postmortem. This suggests that the proteolytic activity of calpain I may contribute to the increased fragility of the myofibril during the 7-day ageing period. However, there was no evidence that spray washing had any effect on calpain I autolysis as there was no significant difference between the relative density of the calpain I in both spray-washed and non-spray washed animals ($p = 0.37$).

Western blotting of two proteins (desmin and troponin T) that are substrates for calpain showed a relationship with tenderness. Troponin T degradation was negatively correlated with pH and shear force. That is, the tough carcasses were those with the least degraded troponin T. This is consistent with the reports of other researchers (Koohmaraie, 1996 Koohmaraie et al., 1987; Geesink and Koohmaraie, 1999a) which demonstrate that post-mortem meat tenderisation is associated with the disappearance of troponin-T and the concomitant appearance of 28-32 kDa polypeptides. However, in this work spray washing had no significant effect on the degradation of troponin T.

Desmin was extensively degraded by seven days post-mortem. The extent of this degradation was related to the MFI and there was a trend towards less degradation in the

meat from those animals which had been spray washed ($p = 0.079$). The concentration of desmin at seven days was between 3 and 17% of the concentration at 24 hours post-mortem. Young et al (1980) observed a similar degradation of desmin during post-mortem storage and it completely disappeared by 42 days of ageing (Geesink et al, 2001).

The curvilinear relationship between meat tenderness and pH_u reported in this experiment is in general agreement with other reports (Purchas, 1990; Watanabe et al, 1996) (see section 2.4.3). The shear force increased as the pH_u progressed from 5.6-5.9 and peaked between pH 5.9 and 6.2. As the pH increased above 6.2 the shear force tended to decrease. Similarly, Watanabe et al (1996) reported that lamb LD muscle with pH_u between 5.8 and 6.2 had the highest shear force values, while those with pH values either above or below this range had a lower shear force. The reason for this variation in tenderness is not understood. The toughness of meat of intermediate pH_u (5.8-6.2) could be a result of shorter sarcomere length and/or reduced proteolysis over this pH range (Yu and Lee, 1986). The results of this experiment showed that the sarcomere length decreased linearly as the pH_u moved towards an intermediate pH range. Thereafter, the sarcomere length increased as the pH_u increased. Thus, the observed toughening of meat of intermediate pH may be due shorter sarcomere length. In support of this, Purchas (1990) showed that shorter sarcomere length occurred in beef with intermediate ultimate pH and may partly explain the toughening of beef with intermediate pH.

It was also found that at one day post-mortem, there was no relationship between pH_u and MFI although it appears that low pH_u (5.47) or high pH_u meat samples have high MFI values. This is consistent with the report of Watanabe et al (1996). These authors found that the MFI, an indicator of proteolysis, was slightly higher for samples whose pH was low (pH 5.4) or high (pH 6.7) compared with the intermediate pH range (pH 5.8-6.2). However, the slight decrease in proteolysis cannot explain the toughness of meat that is usually associated with meat of intermediate pH. Thus, the observed toughness of beef of intermediate pH_u in this research is more likely due to shorter sarcomere length rather than reduced proteolysis.

4.5 Conclusions

It appears that spray-washing cattle does not have a significant effect on the ultimate pH, tenderness or sarcomere length of beef muscles. The limited number of animals in the trial and the large variability in the measured meat parameters from spray washed animals prevented any significant results being obtained. However, there was a trend towards an increased toughness and a decreased sarcomere length as a result of spray washing the cattle. This trend and the observed toughness of meat of intermediate pH are more likely to be related to shorter sarcomeres rather than reduced proteolysis. Furthermore, because the spray-washed animals were not killed immediately after spray washing, the animals may have rested sufficiently to enable the recovery of depleted muscle glycogen to the extent that the ultimate pH was only slightly affected by the treatment.

Chapter 5

5.0 Effect of processing conditions on the tenderness of turkey breast meat

5.1 Introduction

The demand for turkey meat has increased over the last two decades as a result of the increased popularity of further processed turkey products including turkey bacon, ground turkey and turkey burgers (Owens and Sams, 1997). A procedure that allows the early deboning of turkey breast meat was developed at processing plants to increase production efficiency, save energy and labour costs. In New Zealand, the procedure for processing turkey involves withdrawal of turkeys from feed for 12 h and transportation from the production facility to the holding room at the processing plant. The birds are then hung by the shanks on to a moving processing line. Individual birds are electrically stunned (240 V, 160 mA, 60 Hz, 5 s) using an electrical stun-kill knife and killed by exsanguination. After bleeding for 90 seconds, all birds are scalded at 63°C for 45 seconds, defeathered in a rotary drum picker for 30 seconds and the carcasses are cooled under a shower for about 60 seconds. The carcasses are subsequently manually eviscerated. The birds are scalded prior to defeathering to reduce internal haemorrhage during picking.

The carcasses are cooled rapidly using a 2-stage immersion-chilling regime (1st water bath $4 \pm 1^\circ\text{C}$ and a 2nd water bath of $1 \pm 1^\circ\text{C}$ ice-slush; the carcasses are rotated continuously as they pass through the water bath) (Figure 5.1). The time spent in the water baths may vary between 30-40 mins. At the end of this chilling regimen (approx. 45 minutes post-mortem), the carcasses are weighed and placed in a storage basket and held at $0 \pm 1^\circ\text{C}$ for a further 2-3 hours. Then, the carcasses are deboned, packed and exposed to a cold-blast at $-24 \pm 2^\circ\text{C}$ for 2 h. The chilled carcasses are vacuum-packed and subjected to another mini-blast for one hour and then stored at 4°C until transport to retail outlets (Figure 5.1).

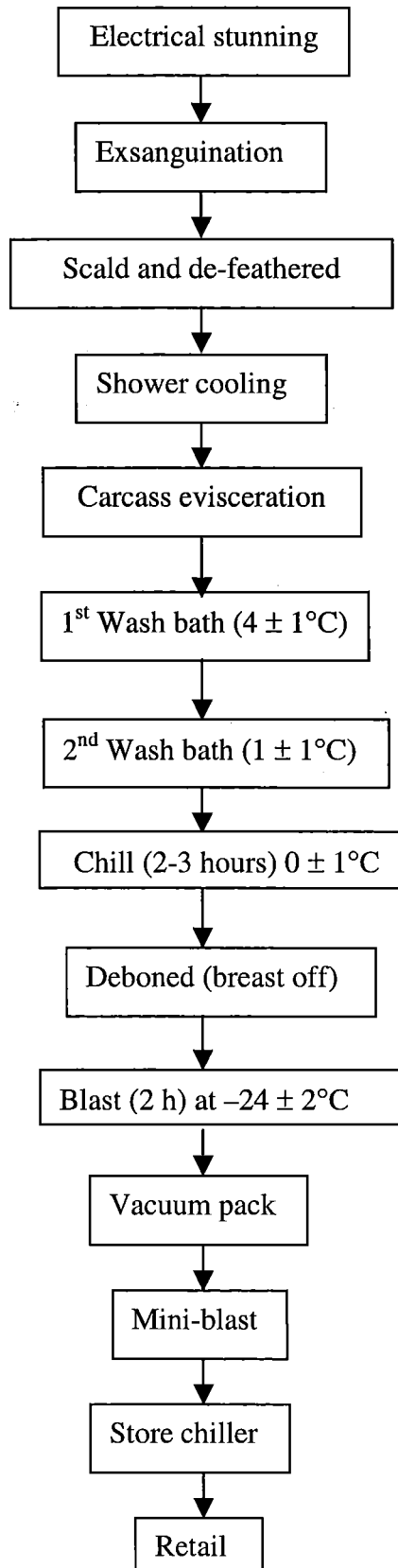


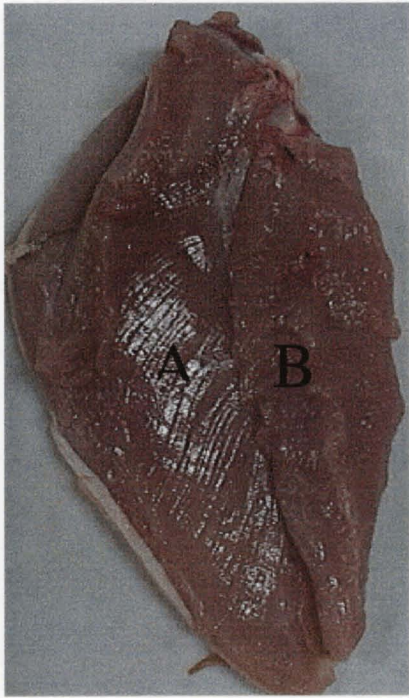
Figure 5.1: Turkey processing procedure as used by the processing plant.

The immersion chilling procedures used in commercial turkey processing plants are designed to rapidly lower carcass temperature to below 4°C. These processes slow the rate of anaerobic glycolysis and the completion of rigor mortis. The processors use this method to slow the pH fall and to avoid pale, soft and exudative (PSE) muscle. This quality defect has resulted in a substantial loss of revenue to turkey meat processors. In addition, the chilling process enables the processors to debone the carcasses early post-mortem without the muscles experiencing 'heat shortening'. However, the immersion of carcasses into ice-cold water bath and the use of blast freezing may induce cold shortening. Thus, the processing procedures may cause toughening of turkey breast meat.

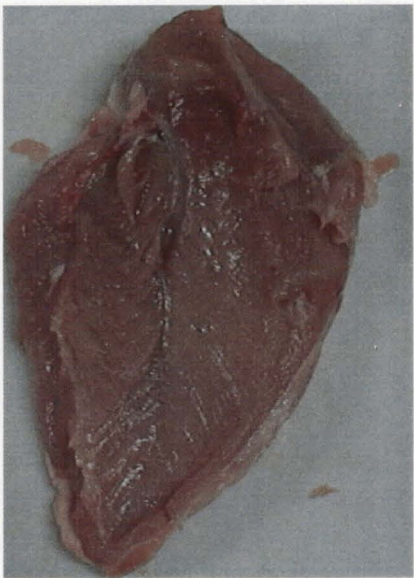
5.2 Experiment 1: Shear force characteristics of turkey breast muscle

5.2.1 Introduction

Experiment one investigated the shear force (tenderness) of turkey breast meat after processing by the existing method (Figure 5.1). This was to investigate the extent of toughness in turkey breast meat which had been reported to occur by the industry (unpublished data). Thirty-turkey breast muscles were processed as described in Figure 5.1 and selected at random to be vacuum-packed using the MIRINZ Cap Tech Machine before overnight storage in the chiller at 4°C. At 24 h, 10 complete turkey breasts were taken from the turkey processing plant to Lincoln University for meat tenderness and pH measurements. To obtain 3 and 7- day samples, another 10 complete breast muscles (from different birds) that had been stored in the chiller at 4°C were taken at 3 and 7 days post-slaughter to determine meat tenderness and pH at Lincoln University. In this study, turkey breast muscle was divided into two parts for analysis, the main muscle and fillet. The fillet is a small flap of muscle attached to the ventral side of the main muscle as shown in Figure 5.2.



Turkey breast muscle



A
Main Muscle



B
Fillet

Figure 5.2: Turkey breast muscle

5.2.2 Results

5.2.2.1 Post-mortem pH of turkey breast muscle

The pH of the main muscle declined significantly from one to three days post-mortem and then remained constant to seven days. In contrast, there was no significant decline in the pH of the fillet from one to seven days post-mortem. Furthermore, the post-mortem pH was significantly higher in turkey breast fillet than in the main muscle at all of the ageing times (Table 5.1).

Table 5.1: Means + standard deviation for shear force, pH and cooking loss of turkey breast main muscle and fillet.

Muscle	Measurement	Time Post-mortem		
		24 h	3 d	7 d
Main muscle	pH	5.96 ^a ± 0.07	5.82 ^b ± 0.09	5.83 ^b ± 0.06
	Shear force (kgF)	3.94 ^a ± 0.12	4.56 ^a ± 0.14	9.00 ^b ± 1.20
	Cooking loss (%)	16.2 ^a ± 1.52	19.1 ^b ± 1.02	17.9 ^{ab} ± 0.98
Fillet	pH	6.01 ^c ± 0.11	5.96 ^d ± 0.05	5.98 ^d ± 0.06
	Shear force (kgF)	3.70 ^a ± 0.23	4.25 ^a ± 0.65	6.00 ^c ± 0.78
	Cooking loss (%)	12.6 ± 1.23	13.0 ± 1.58	15.1 ± 2.56

^{ab} Means in same row with different superscript are significantly different (*p* < 0.01)

5.2.2.2 Meat tenderness

Shear force values for both the main and fillet muscle were higher at three days post-mortem than at 24 hours although the differences were not significant (*p* = 0.12). There was, however, a significant difference between the shear force values at seven days and 24 hours post-mortem for both the fillet and main muscle (Table 5.1) with the shear force being higher at seven days compared to 24 hours or three days post-slaughter.

The shear forces of the fillet and main muscle were similar at 24 hours and three days post-mortem. However, at seven days post-mortem, the shear force was significantly higher for

the main muscle compared to the fillet. The results suggest that turkey breast meat toughens as the meat is aged (Figure 5.3) and the effect is more pronounced in the main muscle.

The cooking loss of the main muscle at 24 hours was significantly lower than at three days but there was no significant difference at seven days (Table 5.1). The cooking losses of fillets were not significantly different at any of the ageing times. The main muscle showed higher cooking losses at all ageing times compared to the fillet.

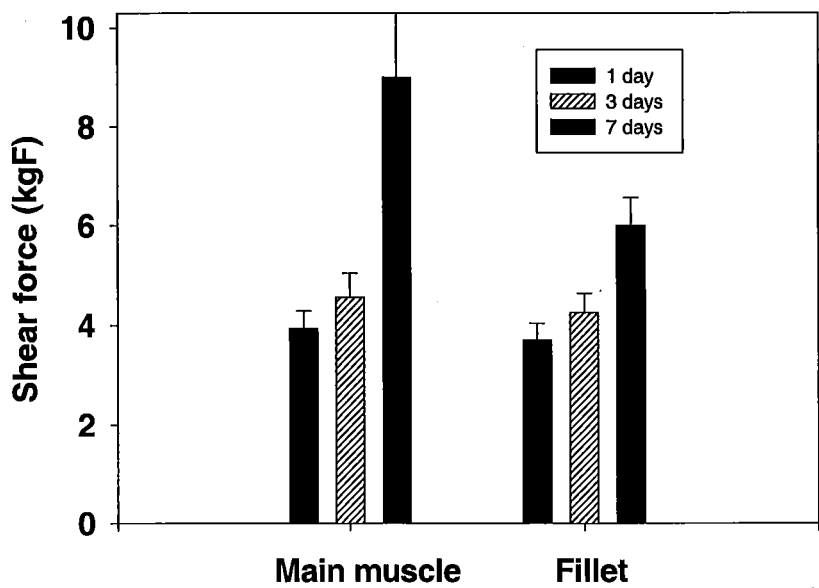


Figure 5.3: Tenderness (kgF) and error bars of cooked turkey breasts main muscle and fillets.

5.2.3 Conclusion

According to unpublished industry reports, consumers’ perceive that turkey meat has become tougher. The reported results support the consumers’ observation that turkey meat usually reaches consumers 3-7 days post-slaughter. The results of this experiment suggest that turkey breast meat toughens with ageing although different muscles were used at the different post-mortem times. The toughening of the turkey breast meat in this experiment may be due to muscle shortening but further investigation is needed to validate these findings.

5.3 Experiment 2: Influence of chilling regime on the tenderness of turkey breast meat.

5.3.1 Introduction

As mentioned previously, the toughness of turkey breast meat may be influenced by a number of post-slaughter factors including: (i) the extent of electrical stunning, (ii) the rate of temperature fall in the chilled wash tumblers (iii) the time in the chilled wash tumblers, (iv) the time exposed to blast freezing and (v) the time at which the carcasses are deboned post-slaughter.

By deboning the muscle early, the processors are able to separate and discard the carcasses. This helps to reduce the labour, energy costs and cooler space required for ageing whole carcasses. However, the harvesting of broiler breast meat before the completion of rigor mortis has been shown to toughen meat (Lyon et al, 1985). The objectives of this experiment were to investigate: (i) the impact of lowering the temperature using an extended ice-slush wash and (ii) the effect of 3 h post-mortem deboning versus leaving the muscle to be aged on bone on the tenderness of turkey breast meat.

5.3.2 Experimental design

Twelve turkeys of similar weight (8.5-9.0 kg) were selected from the same turkey supplier and processed according to the turkey plant processing procedure as described in Figure 5.1. After the shower cooling (15 min post-mortem), the temperature and the pH of each turkey breast was determined. The carcasses were randomly divided into two groups, A and B (n = 6 in each group). Carcasses in group A were passed through the two chill water baths as described in section 5.1 ('bath once only' group). Carcasses of group B were passed through the second ice-slush bath a second time ('bath twice' group). The temperature and pH of all the carcasses were measured after the wash baths and prior to being placed in the chiller (maintained at 0°C) for approximately 2 1/2 hours. After chilling, both the right and left breast muscles were removed from three of the carcasses of group A ('off bone'). The breast muscles were left intact (on the barrel) in the three remaining carcasses ('on bone'). Carcasses in group B were treated the same as group A

carcasses (Figure 5.4). All the meat samples originating from the two groups of carcasses were cold-blast treated as described in section 5.1.

At 24 hours post-slaughter, all the remaining breast muscles from group A and B were removed from the bone. All the main breast muscles, including those removed at three hours post-mortem, were split in half. One half was used to determine the pH, meat tenderness and some biochemical parameters. The other half was vacuum-packed and aged at 4°C for a further six days. At the end of the 7-day post-slaughter ageing period, the meat quality attributes measured on the 24-hour samples were also determined on the 7-day post-slaughter samples. In this and subsequent experiments, the tenderness attributes and biochemical parameters were determined on the main muscle of turkey breast meat for all the treatments and a paired t-test was used to analyse the data.

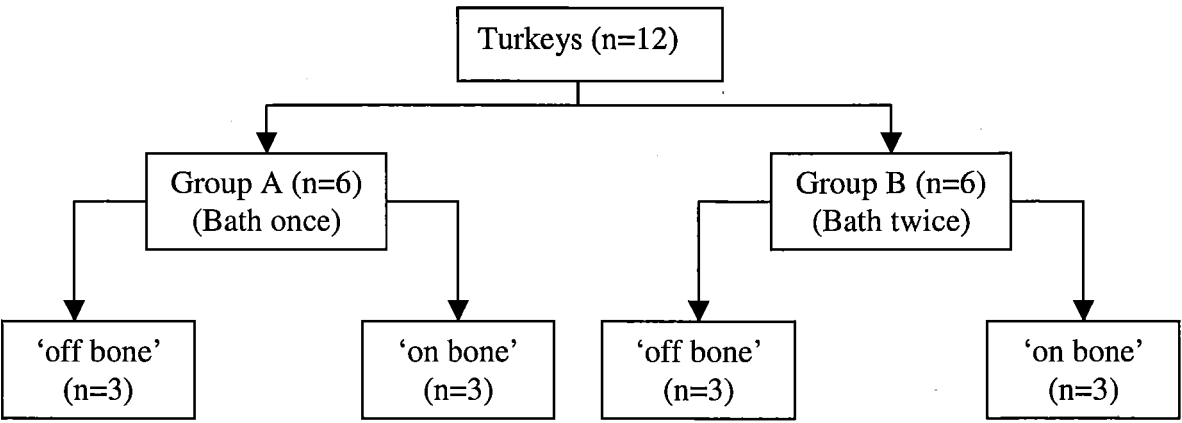


Figure 5.4: Design of experiment 2

5.3.3 Results

5.3.3.1 Temperature and pH conditions of turkey breast muscle at various times post-mortem

The mean temperature of the carcasses at 15 minutes post-slaughter (after being scalded and defeathered) was 35.5 °C. There was a rapid drop in temperature between 15 minutes and 45 minutes post-mortem (after the 1st and 2nd bath) in both treatments (Figure 5.5). However, after 45 minutes and 180 minutes post-mortem, the fall in temperature of the carcasses in both treatments was virtually complete and the carcasses attained their desirable temperatures. This result was expected because of the cooling effects of the ice-

slush water bath. In addition, after 90 minutes the mean temperature of those carcasses that went through the bath once was always significantly higher than those that went through the bath twice (Figure 5.5). This difference remained even after deboning and cold blast treatment of the carcasses (Table 5.2).

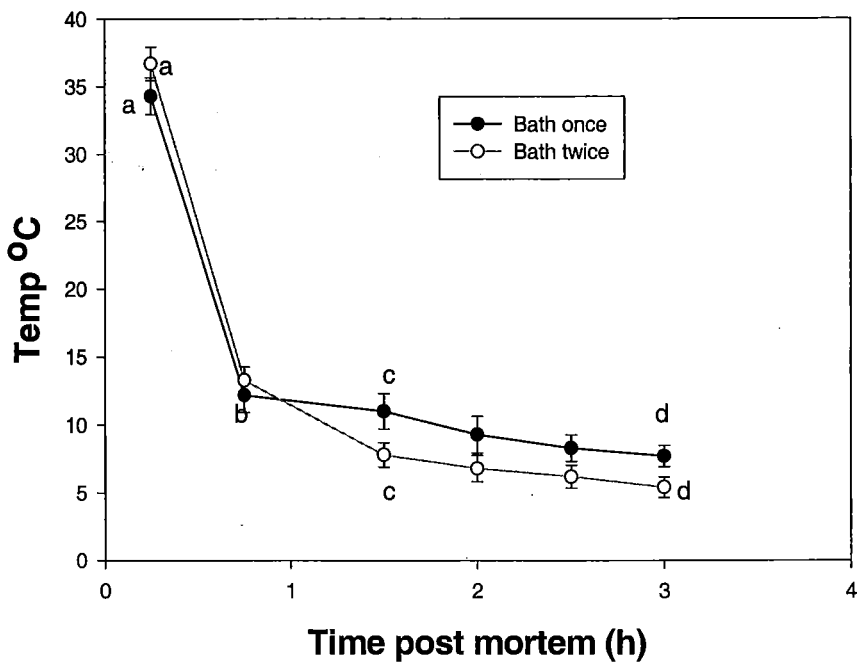


Figure 5.5: Post-mortem temperature profile (a = before 1st bath, b= after 2nd bath (45mins), c = after passing through the 2nd bath a second time and d = after 1st chill at 0°C, respectively)

Table 5.2: Mean + standard deviation of temperature and pH of ‘off bone’ and ‘on bone’ muscles from ‘bath once’ and ‘bath twice’ carcasses.

Time PM	After deboning		After blast		After chilled storage	
	(3.5 h)		(5.5 h)		24 h	
Bath once	pH	Temp °C	pH	Temp °C	pH	Temp °C
Off Bone	6.10±0.17	9.1 ^a ±0.22	6.07±0.10	4.2 ^a ±0.05	5.90 ^a ±0.19	4.2±0.04
On Bone	6.35±0.15	8.7 ^a ±0.18	5.84±0.11	4.5 ^a ±0.28	5.89 ^a ±0.23	4.1±0.09
Bath Twice	pH	Temp °C	pH	Temp °C	pH	Temp °C
Off Bone	6.27±0.16	7.0 ^b ±0.44	5.95±0.09	2.2 ^b ±0.47	5.91 ^a ±0.30	4.7±0.12
On Bone	6.29±0.10	5.9 ^b ±0.40	5.91±0.04	2.4 ^b ±0.15	5.83 ^b ±0.16	4.3±0.14

^{ab} Means in the same column with different superscript are significantly different, p < 0.05

The post-mortem pH profile of turkey breast muscle after the two bathing regimes is shown in Figure 5.6. There was a gradual decline in muscle pH up to 1.5 hours post-mortem in both the carcasses that were passed through the ice-water bath ‘twice’ and ‘once’. At 1.5

hours post-slaughter, those carcasses which passed through the 2nd water bath once produced a lower pH than those carcasses which passed through the 2nd water bath twice. From 1.5 to 2.5 hours post-mortem, there was a rapid decline in pH for those that went through the 2nd bath twice. However, after 2.5 hours post-mortem, the fall in pH was slower through to the ultimate pH. The decline in pH after 1.5 hours post-mortem for those carcasses that went through the bath once also followed the same trend as those that went through the bath twice.

At all times post-mortem (15–180 mins), the pH of those carcasses which passed through the bath once tended to be lower than those that passed through twice, although the differences in the pH measurements were not significant ($p= 0.067$) (Figure 5.6). Between 15 mins and three hours post-mortem, the pH of the breast muscle had declined from 6.43 to 6.18 for those that went through the second bath once, and from 6.46 to 6.23 for those carcasses which went through the 2nd bath twice.

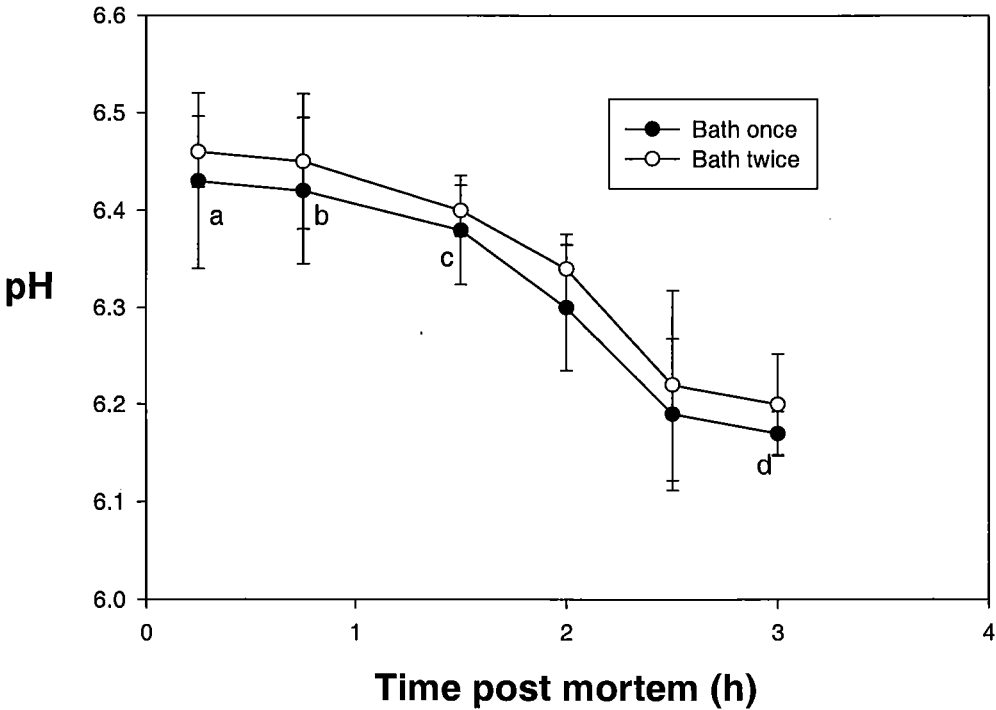


Figure 5.6: Post-mortem pH decline in turkey breast muscle (a = before 1st bath, b= after 2nd bath (45mins), c = after passing through the 2nd bath a second time and d = after 1st chill at 0°C).

After deboning (3 1/2h post-mortem) (Table 5.2), the mean pH of the breast muscle ‘off bone’ from those that passed through the bath twice was 6.10 while the breast muscle ‘off

bone' from carcasses which passed through the bath once had a mean pH of 6.27. After chill and blast treatment (5.5 h post-mortem), the mean pH of the 'off bone/bath once' breast muscle fell by 0.03 units compared to 0.51 units of the 'on the bone/bath once' muscle. In the second group (bathed twice), the pH of the 'off bone' muscle fell 0.32 units after chill + blast treatment and the 'on bone' muscle had a pH decline of 0.38 units (Table 5.2).

At 24 hours post-mortem, there was no significant difference between the muscle pH within the treatments of group A ('off bone/bath once' and 'on bone/bath once'). In contrast, the muscle pH from group B ('off bone/bath twice' and 'on bone/bath twice') differed significantly at 24 hour post-slaughter. The muscle that was left 'on bone' and which received the bath treatment twice had the lowest pH (5.83) at 24 h.

5.3.3.2 Meat quality characteristics

The effects of the bathing regime on the tenderness and biochemical attributes of turkey breast meat are shown in Table 5.3. At 24 hours post-slaughter, the type of bathing treatment had no significant effect on meat tenderness irrespective of whether the muscles were excised from the bone after 3 hours post-mortem and aged 'off' ('off bone') or aged 'on' the bone for 24 hours ('on bone'). But after seven days of ageing, there was a significant difference between the tenderness of meat from 'off bone' and 'on bone' in both bathing treatments. In both chilling regimes, the shear forces of muscles from 'off bone' were higher than those from 'on bone' at seven days post-mortem. In addition, the shear force of meat from 'on bone'/'off bone' ('bath once') was significantly lower than that from 'on bone'/'off bone' ('bath twice')(Table 5.3).

Table 5.3: Means with standard deviations for shear values, MFI, sarcomere length and cooking loss of turkey breast meat for the bath treatment.

Measurement	Time PM	Bath once		Bath twice	
		Off Bone	On bone	Off Bone	On bone
Shear force (kgF)	1d	5.69 ^a ±1.48	4.05 ^{ab} ±0.98	5.70 ^a ±1.81	4.17 ^a ±0.87
	7d	6.96 ^a ±1.04	3.88 ^b ±0.25	8.74 ^c ±1.40	4.65 ^a ±0.51
Cooking loss (%)	1d	15.1 ± 1.36	16.5 ± 0.95	16.4 ± 1.96	17.0 ± 2.01
	7d	17.2±2.13	14.5±1.36	15.8±0.98	18.3±1.24
MFI	1d	58.8±7.83	59.0±10.1	67.5±5.64	63.8±1.56
	7d	65.2±10.8	68.0±12.0	52.1±2.00	64.2±2.12
Sarcomere length (µm)	1d	1.90 ^a ±0.11	2.02 ^a ±0.09	1.90 ^a ±0.20	1.94 ^a ±0.04
	7d	1.77 ^b ±0.08	1.87 ^{ab} ±0.08	1.74 ^b ±0.10	1.79 ^b ±0.06

^{abc}Means in the same row with different superscript are significantly different, p< 0.05

In contrast to the results obtained in Experiment 1, the mean shear values were similar at day 1 and seven days post-slaughter except for the muscles from ‘off bone’/‘bath twice’, which tended to be higher at seven days post-mortem (p = 0.056). Thus, the tenderness of turkey meat did not improve after extended ageing. Breast muscles deboned at three hours post-mortem from the individual carcasses showed wide variations in shear force values compared to breast muscles left on the bone for 24 hours. This is evident in the large standard deviation Figures (Table 5.3). Figure 5.7 highlights this variability. Carcasses 1 and 6 showed large differences in shear force values between the muscle deboned at three hours post-mortem and muscle aged on the bone for 24 hours post-mortem whilst there were only small differences with time in the other four carcasses.

5.3.3.3 Myofibrillar fragmentation index (MFI) and sarcomere length

The effect of the treatments on the myofibrillar fragmentation index (MFI), which is a measure of proteolysis, is shown in Table 5.3. There was no evidence that the treatments had any effect on MFI. Surprisingly, although slightly higher, the MFI at seven days was not significantly different from that at 24 hours post-mortem. A high MFI indicates a high rate of proteolysis. Over the six days ageing period, the MFI values suggest that the meat did not undergo any proteolysis.

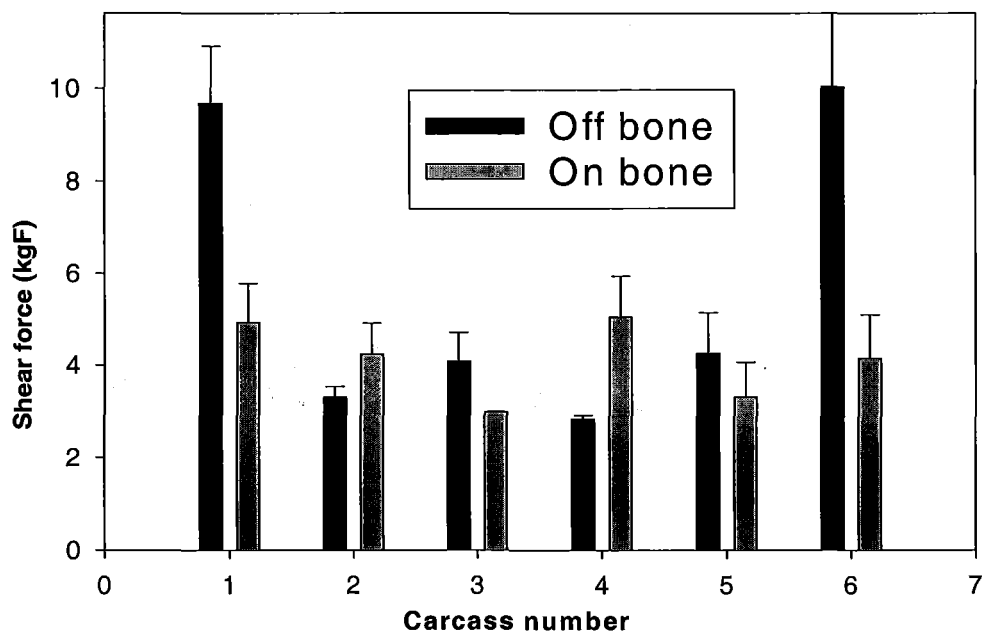


Figure 5.7: *Shear force of muscle ‘off bone’ and ‘on bone’ at 24h post-mortem from the same carcass.*

The sarcomere length of the muscle fibres was measured. At day 1 post-mortem, the sarcomere lengths were relatively long (1.90 – 2.02 μm) and the sarcomere lengths were unaffected by the treatments with a mean of 1.90 and 1.98 μm for muscles off bone and on bone, respectively. Although the treatments had no effect on the sarcomere lengths at seven days, the sarcomere lengths for all the treatments were shorter after seven days of ageing compared to the sarcomere lengths at day 1 post-mortem. For those muscles ‘on bone’ (bathed once), ageing the muscles for a further six days had no significant effect on the sarcomere length.

5.3.4 Conclusion

There was evidence that double washing turkey carcasses resulted in a tendency for the turkey breast meat to toughen. However, leaving the muscle to age on the bone for 24 h improved its overall tenderness. In other words, the deboning of turkey breast muscles 3 h post-mortem allowed the muscles to contract as shown by the shorter sarcomere lengths in these muscles. The toughening of turkey meat in this experiment may be due to cold shortening of the deboned muscles subjected to the blast freeze temperature of $-24\text{ }^{\circ}\text{C}$ for two hours.

5.4 Experiment 3: Effect of electrical stunning intensity on the tenderness of turkey breast meat

5.4.1 Introduction

In experiment 2, there was evidence that suggested that muscles deboned at three hours post-mortem tended to have shorter sarcomere lengths compared to muscles de-boned at 24 h post-mortem. The cause of this contraction may be due to (i) the rate of cooling associated with the chilling regime (ii) the effects of the cold-blast treatment and (iii) the electrical stunning conditions. In this experiment, the effects of electrical stunning and an extended ageing period of up to seven days on the tenderness of breast muscles 'off the bone' at 24 h and 'on the bone' until seven days post-mortem were investigated. Electrical stunning used at the processing plant is in the form of an immobiliser, which reduces struggling and distress in birds during exsanguinations. Electrical stunning also shortens bleed-out time and ensures the death of the bird prior to scalding. Electrical stunning not only renders the bird unconscious but delays post-mortem muscle glycolysis (through reduced birds' struggling) and increases meat tenderness (Murphy et al, 1988). However, electrical stunning may also reduce the meat quality if the current applied is too low or too high. Since electrical stunning may affect meat quality, the effects of 'double electrical stunning' and chilling regime on meat tenderness were examined in this section of work.

5.4.2 Experimental design

Twenty-four birds were selected within a uniform weight range from a population of 17-week old turkeys at a local processing plant. The individual birds were hung on shackles by the shanks and tagged. The birds were divided into two groups (n = 12 each). The first group was electrically stunned by the same procedure as described in section 5.1 and killed by exsanguination ('Single stunned'). In the second group, individual birds were electrically stunned as described for the first group. However, they were re-stunned a second time before being killed by exsanguination (n = 12) ('Double stunned').

Thereafter, all the turkeys were treated in the same manner as described in section 5.1 until after the first chill (i.e. after 2 ½ h in the 0°C chiller). All the breast muscles were left on

the bone for 24 hours, but after the first chill, six of the carcasses in group A ('Single stunned') and six in group B ('Double stunned') were cold-blasted at -24°C for two hours ('chill+ blast'). The remaining twelve carcasses remained in the chiller at 0°C for the two hours ('chiller only') (Figure 5.8). The temperature and pH of the breast muscle was measured after the chilling regimen. Each carcass was vacuum packed and placed in a box. The boxes were again subjected to a cold blast before being stored in a chiller at 4°C until 24 hours post-mortem.

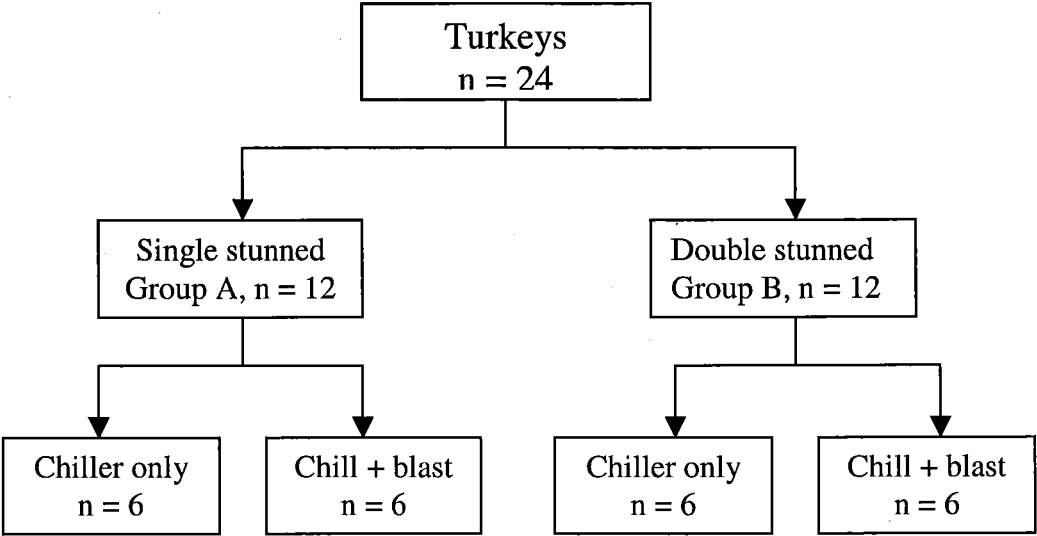


Figure 5.8: The design for experiment 3

At 24 h post-mortem, the right breast muscle was removed from all the carcasses and the main muscles were split into two halves. One half was used for 24 h pH, MFI, sarcomere length, tenderness and cooking loss determinations. The other half of the main muscle was vacuum packed and aged at 4°C for a further six days. The carcass with the left breast muscle was vacuum packed and aged for a further six days. After the ageing period, the muscles were removed and the sarcomere length, pH, MFI, tenderness and cooking loss determined on all the samples.

5.4.3 Results

5.4.3.1 Post-slaughter temperature and pH decline

The post-mortem rate of temperature decline was similar for the ‘single’ and ‘double’ stunned carcasses. The first temperature measured for the ‘single’ and ‘double’ stunned carcasses at 15 minutes post-slaughter was 32.5°C and 33.5°C respectively. At one hour post-mortem the temperatures had fallen to 8.2°C and 8.3°C respectively as a result of the cooling effect of the ice-slush bath (Figure 5.9). All the carcasses were placed in a chiller at 0°C for two hours, and at the end of this chilling regime, the mean internal muscle temperature for the ‘double’ and ‘single’ stunned carcasses were similar. In both stunning treatments, the mean internal muscle temperature at six hours post-mortem of turkey carcasses that were subjected to ‘chill + blast’ treatment was significantly lower than that from carcasses that were ‘chilled only’ immediately after the chilling regime (Table 5.4).

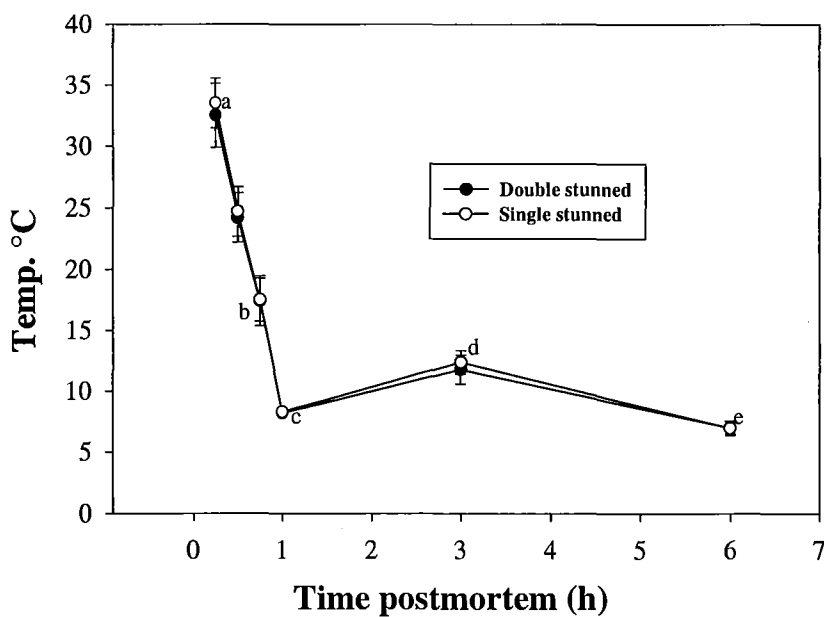


Figure 5.9: Post-mortem temperature fall in muscles from pre-slaughter single and double stunned carcasses (a = before 1st bath, b = after 2nd bath (45mins), c = before 1st chill, d = after 1st chill at 0 °C and e = after cold blast).

Table 5.4: Mean + standard deviation of temperature and pH of double and single stunned turkey carcasses after chill or blast treatments.

Time post-slaughter	After deboning (3h)		After blast or chill (6h)		After chilled storage 24h	
	pH	Temp °C	pH	Temp °C	pH	Temp °C
Double stunned						
Chill only	6.10 ^a ±0.21	11.8±1.27	5.92±0.13	7.92 ^a ±0.57	5.90±0.11	4.2±0.03
Chill+ blast	5.90 ^b ±0.11	11.8±1.24	5.88±0.09	6.08 ^b ±1.07	5.87±0.03	4.1±0.08
Single stunned						
Chill only	6.05 ^a ±0.07	12.2±1.23	5.96±0.14	7.98 ^a ±0.76	5.85±0.08	4.5±0.07
Chill+ blast	6.18 ^a ±0.16	12.6±0.68	6.00±0.20	6.00 ^b ±1.81	5.90±0.06	4.3±0.10

^{ab} Means in the same column with different superscripts are significantly different, p < 0.05

Figure 5.10 shows the post-mortem pH decline in turkey breast meat from carcasses that were ‘double’ or ‘single’ electrically stunned pre-slaughter. The first post-slaughter pH was measured at 15 minutes post-mortem, as this was the earliest possible time to access carcasses at the processing plant. At 15 min, 30 min, 45 min and 60 min post-slaughter, the pH of the ‘double’ stunned carcasses were significantly lower than that of ‘single’ stunned carcasses (p = 0.001, 0.019, 0.012 and 0.018, respectively).

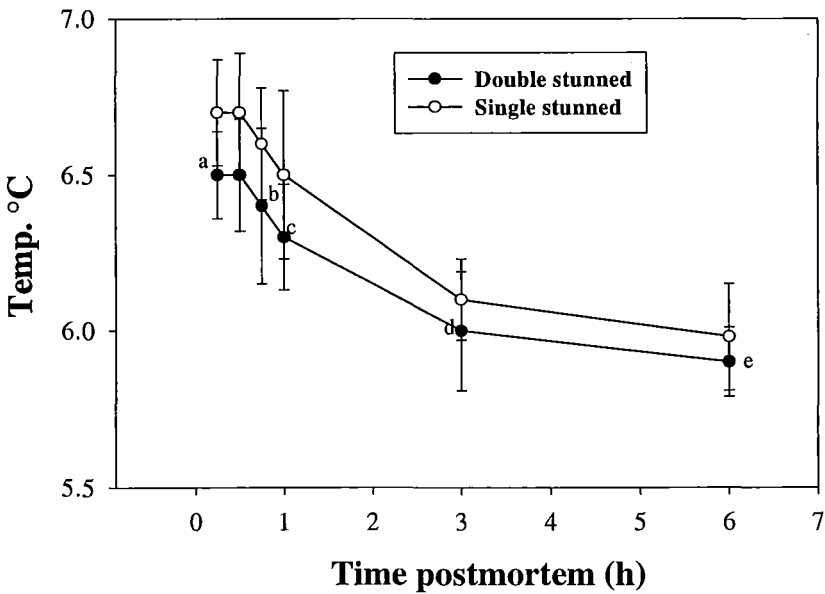


Figure 5.10: Post-mortem pH decline in muscles from pre-slaughter single and double stunned carcasses (a = before 1st bath, b = after 2nd bath (45mins), c = before 1st chill, d = after 1st chill at 0 °C and e = after cold blast).

However, at six hours and 24 hours post-slaughter, the pH of the ‘double’ and ‘single’ stunned carcasses were similar irrespective of the chilling treatments (Table 5.4).

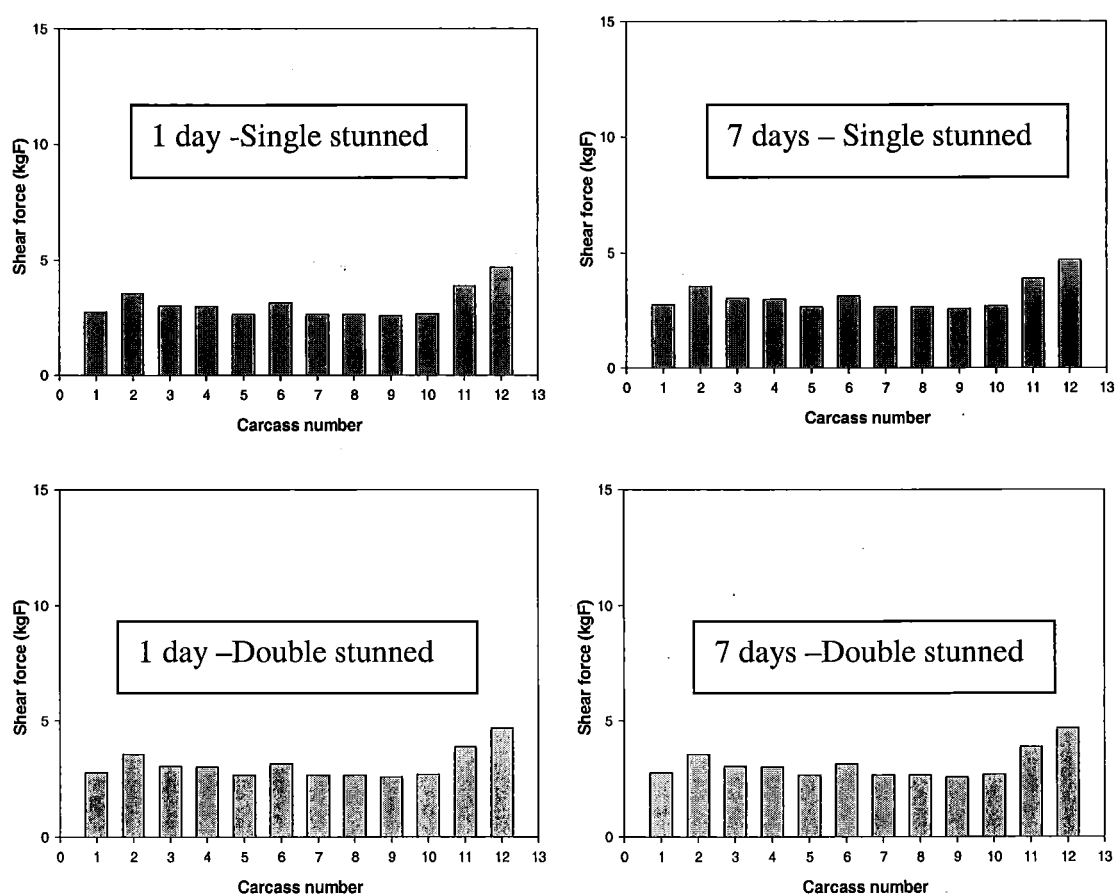


Figure 5.11: Shear force for muscle from double or single stunned carcasses at one day and seven days post-mortem.

5.4.3.2 Breast meat Tenderness

In this experiment, all the breast muscles were aged on the bone for at least 24 hours. At day 1 post-mortem, there was no significant difference between the shear forces of the breast muscle from ‘double stunned’ (‘chiller only’ or ‘chill + blast’) and ‘single stunned’ (‘chiller only’ or ‘chill + blast’). Interestingly though, all the meat from the treatments were tender (Figure 5.11). This indicates that the toughening effects observed in experiment 2 could be the result of cold shortened muscles since in this experiment all the muscles were aged on bone which provided restraint and prevented muscle contraction. Although, the treatments produced slight differences at 24 hour and day 7 post-mortem in shear force values, there was no evidence to suggest that removing muscle from the bone at 24 hours

and ageing it for an additional six days improved the tenderness of the breast muscle (Table 5.5). In contrast, the breast muscle that was allowed to age on the bone for seven days before removing it from the bone showed improved tenderness values (Table 5.6). This means that allowing the muscle to age ‘on bone’ for seven days improves the tenderness of meat.

The cooking loss showed no significant difference in the treatments. However, the meat from carcasses that were ‘single stunned’ tended to have higher cooking loss than those that were ‘double stunned’, regardless of whether the carcasses was ‘chilled only’ or ‘chilled + blast’.

Table 5.5: Influence of pre-slaughter electrical stunning on muscle pH, shear force, cooking loss, sarcomere length and MFI for muscle ‘off bone’ at 24h and aged for seven days (mean \pm standard deviation).

Measurement	Day (PM)	Double stunned		Single stunned	
		Chill only	Chill +Blast	Chill only	Chill +Blast
pH	1	5.90 \pm 0.12	5.88 \pm 0.03	5.85 \pm 0.08	5.90 \pm 0.06
	7	5.86 \pm 0.08	5.86 \pm 0.05	5.85 \pm 0.10	5.93 \pm 0.07
Shear force (kgF)	1	3.02 \pm 0.32	3.19 \pm 0.89	3.02 \pm 0.54	3.45 \pm 0.50
	7	2.91 \pm 0.39	3.45 \pm 1.41	3.18 \pm 0.50	2.75 \pm 0.66
Cooking loss (%)	1	16.6 \pm 1.30	16.6 \pm 1.33	17.5 \pm 1.63	17.4 \pm 1.45
	7	16.9 \pm 0.98	16.8 \pm 1.22	17.2 \pm 1.02	17.0 \pm 1.98
Sarcomere length (μ m)	1	1.95 \pm 0.12	2.03 \pm 0.08	1.90 \pm 0.07	1.97 \pm 0.05
	7	1.96 \pm 0.13	2.01 \pm 0.11	1.92 \pm 0.16	1.98 \pm 0.06
MFI	1	58.2 ^a \pm 7.70	44.9 ^b \pm 1.09	53.0 ^a \pm 1.01	48.5 ^b \pm 1.04
	7	65.4 ^a \pm 3.99	59.9 ^a \pm 10.7	61.0 ^a \pm 8.39	65.2 ^a \pm 4.03

^{ab} Means in the same row with different superscript are significantly different, $p < 0.01$

5.4.3.3 Structural and biochemical changes associated with post-mortem ageing

Unlike the second experiment, the length of the sarcomere was similar in all treatments and at all post-mortem ageing times. The sarcomere lengths of all the muscles from the carcasses of the treatments were long and ranged between 1.90 – 2.04 μ m

Table 5.6: Influence of pre-slaughter electrical stunning on muscle pH, shear force, cooking loss, sarcomere length and MFI for muscle aged on bone for seven days (Mean \pm standard deviation).

	Day (PM)	Double stunned		Single stunned	
		<i>Chill only</i>	<i>Chill + Blast</i>	<i>Chill only</i>	<i>Chill + Blast</i>
pH	7	5.80 \pm 0.11	5.80 \pm 0.09	5.80 \pm 0.03	5.90 \pm 0.12
Shear force (kgF)	7	2.98 \pm 0.23	2.40 \pm 0.02	2.64 \pm 0.06	2.24 \pm 0.05
Cooking loss (%)	7	17.7 \pm 1.20	15.3 \pm 1.99	14.3 \pm 1.36	16.6 \pm 0.98
Sarcomere length (μm)	7	1.90 \pm 0.01	2.04 \pm 0.08	1.92 \pm 0.08	1.96 \pm 0.10
MFI	7	80.2 ^a \pm 3.69	78.6 ^a \pm 2.56	65.3 ^b \pm 1.59	64.1 ^b \pm 1.89

^{ab} Means in the same row with different superscript are significantly different, $p < 0.01$

The extent of proteolysis, as measured by the MFI, was higher in meat from ‘chilled only’ muscle compared to ‘chill + blast’ meat at 24 h and seven days post-mortem (Table 5.5). The result also showed that the MFI of ‘chill + blast’ was significantly lower than the MFI from ‘chill only’ carcasses, irrespective of stunning treatment, at 24 hours post-mortem. However, at seven days post-mortem, the MFI was similar for all the muscles and was independent of stunning treatment and chilling regimes. In the case of muscles aged ‘on bone’ for seven days, the MFI results followed a different trend (Table 5.6). It was evident that double stunning had a significant effect on the fragmentation of the muscle myofibrils compared to ‘single’ stunning. However, within each stimulation treatment, ‘chill only’ or ‘chill + blast’ had no effect on MFI.

5.4.4 Conclusions

The application of double stunning to turkey carcasses did not improve the tenderness of breast muscles aged on the bone. The results of this experiment also indicated that the cold-blast treatment does not influence the tenderness of turkey breast meat provided the muscles remain on the bone. This section of research also confirms that leaving the muscle on the bone during ageing improved the overall tenderness of turkey breast muscle.

5.5 Experiment 4: Influence of pre-rigor deboning on the tenderness of turkey breast muscle

5.5.1 Introduction

It was observed in experiment 2 that deboning of turkey breast meat three hours post-mortem toughened the breast muscle. It was also evident in experiment 3 that leaving the muscle to age on the bone improved the tenderness of meat. From experiment 2 and 3, it also appears that double washing and double stunning did not have any beneficial effects on the tenderness of turkey breast meat. The specific objectives of this experiment were to investigate (i) the existing processing procedure (as described in section 5.1) against 'on bone' ageing (ie ageing muscles on bone for at least 24 h before deboning) and (ii) the effect of 'chill only' versus 'chill + blast' on the tenderness of turkey breast meat.

5.5.2 Experimental design

In this experiment, forty-eight birds were selected with a uniform weight range (9.0-10.0 kg) from a population of 17-week old turkeys at a local processing plant. The birds were processed according to the conventional method as described in section 5.1. However, after the first chill at 0°C for 2 h the carcasses were divided into two groups, A and B (n = 24 in each group) (Figure 5.12). The right breast muscle of all the carcasses of group A was removed while the left muscle was left on the bone. The right muscles were labelled 'off bone' and the left 'on bone'. Both the breast muscles from the group B carcasses were left intact and labelled 'intact muscle on bone'. After the pH and temperature were measured, half the muscles from 'off bone' (n = 12), half the muscles left 'on bone' (n = 12), and half the intact carcasses (n = 12) were cold-blasted at -24°C for two hours ('chill + blast'). The other breast muscles from 'off bone', 'on bone' and 'intact on bone' were returned to the chiller and maintained at 0°C for two hours ('chiller only'). Subsequently, the pH and temperature were again measured. The samples were then vacuum packed, placed in a storage box and subjected to the cold-blast treatment. The samples were then stored in a chiller until 24 hours post-mortem.

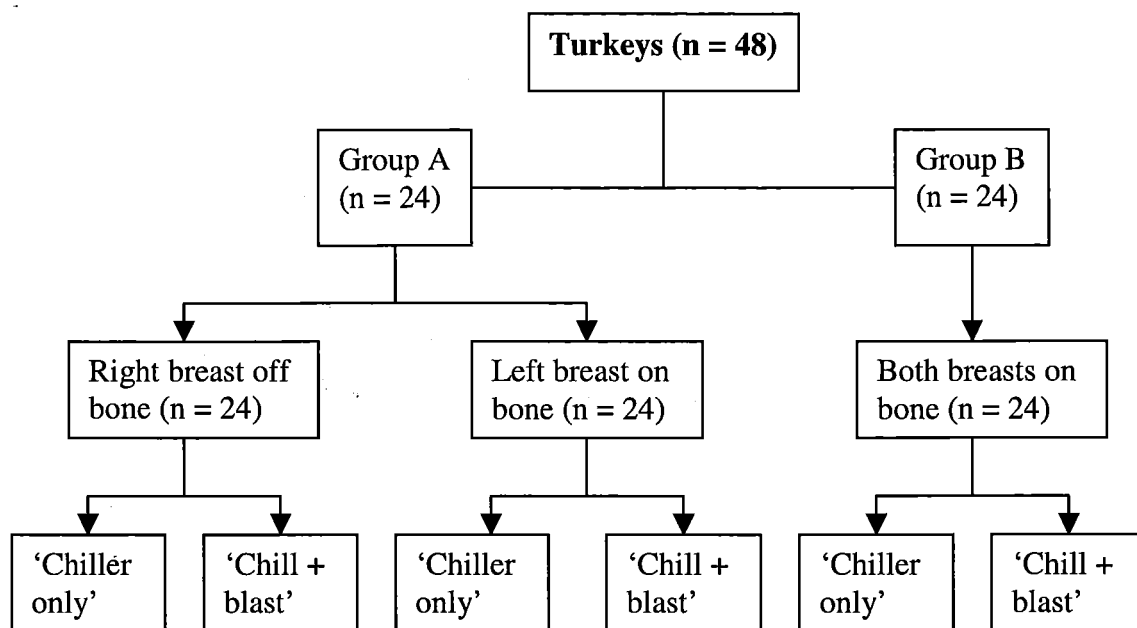


Figure 5.12: The design for experiment 4

At 24 hours post-mortem, the samples were taken from the processing plant to Lincoln University and the main muscles from the 'off bone' were split into two equal parts with a knife. One half was used for 24-hour pH, temperature, sarcomere length, MFI and meat tenderness determinations. The remaining half was vacuum packed and aged at 4°C for a further six days. This sample was used to determine seven days post-mortem meat tenderness. The left breast muscle 'on bone' was removed and the pH and temperature measured (24-hour pH). Once again, the main breast muscle was split in half. One half was used for the determination of 24-hour meat quality parameters and the other half was aged for a further six days as described above. This was then used to determine the meat quality attributes as in the previous group.

In the case of group B, the right breast muscles were removed from all the carcasses, and the left breast muscle, which remained on the bone, was vacuum-packed and aged for seven days. The right muscles were split in half, one half vacuum packed and aged for a further six days and the other half used to determine the 24 hour meat tenderness, MFI, sarcomere length and cooking loss. The breast muscles aged on the bone were removed at seven days post-mortem. The pH, meat tenderness, cooking loss, sarcomere length and MFI were determined for all the main muscles.

5.5.3 Results

5.5.3.1 Temperature and pH profile of turkey breast meat

The mean temperature of all the carcasses (n = 48) at 30 minutes post-slaughter was 36.5°C. This was after scalding and defeathering the birds, carcass shower cooling and evisceration. The first water bath lowered the mean internal temperature of the breast muscles from all the carcasses to 25.2°C. The second bath (ice slush bath) decreased the mean temperature to about 11.0°C over 30 minutes. However, over the next two hours after passing through the second water bath and after the first chill at 0°C, the fall in the breast muscle temperature was about 4°C. The rapid temperature fall between 30 minutes and 75 minutes post-slaughter was expected because of the cooling effect of the ice water bath on the carcasses (Figure 5.13).

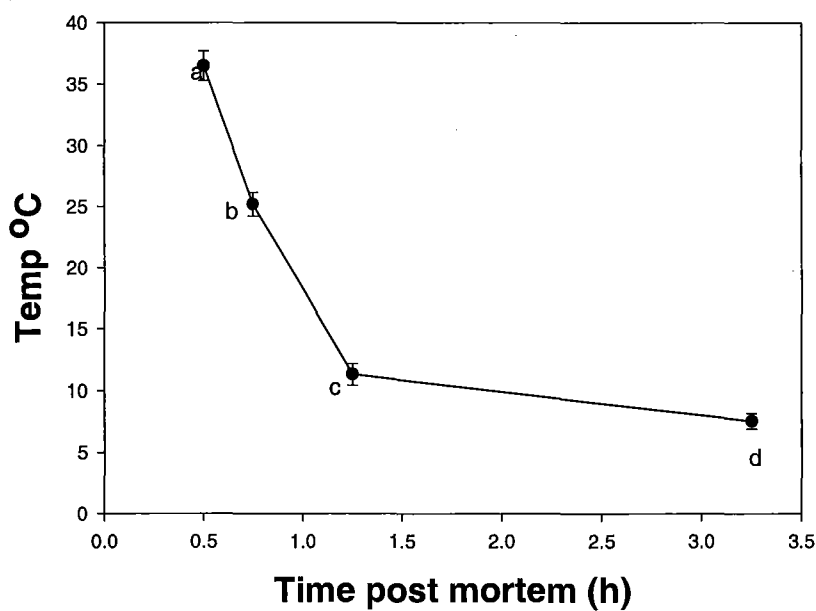


Figure 5.13: Post-mortem temperature decline in turkey breast muscle (a = before 1st bath, b = after 1st bath (45mins PM), c = after 2nd bath (75mins PM), d = after 1st chill at 0°C).

The ‘chill + blast’ treatment (n = 24) significantly reduced the internal breast muscle temperatures irrespective of whether the muscle was left on the bone or removed from the bone. Whereas, the chiller did not have any significant effect on the internal temperature of the breast muscles that remained in the chiller (0°C) for a further 2 hours. In both ‘off bone’

(n = 24) and ‘on bone’ muscles (n = 24), the internal temperature of the ‘chill only’ (n = 24) was significantly higher than the temperature of the ‘chill + blast’ muscles (Table 5.7).

Table 5.7: Mean + standard deviation of temperature and pH of ‘off bone’ and ‘on bone’ muscles after chill or chill +blast treatments.

Turkey breast Muscle Treatment	After 1 st chill at 0°C (3 h)		After boning, and chill or chill+ blast (6 h)		After chill storage 24 h	
Main ‘Off bone’	pH	Temp °C	pH	Temp °C	pH	Temp °C
Chill only (n=12)	5.94±0.14	7.9±0.58	6.11±0.13	7.9 ^a ±0.32	5.93±0.09	4.3±0.02
Chill+ blast (n=12)	6.00±0.24	7.6±0.70	6.06±0.09	3.3 ^b ±0.14	5.92±0.10	4.0±0.06
Main ‘On bone’	pH	Temp °C	pH	Temp °C	pH	Temp °C
Chill only (n=12)	5.94±0.14	7.9±0.58	6.04±0.12	7.6 ^a ±0.36	5.97±0.11	4.4±0.07
Chill+ blast (n=12)	6.00±0.24	7.6±0.70	5.95±0.09	3.2 ^b ±0.11	5.91±0.06	4.4±0.09

^{ab} Means in the same column with different superscripts are significantly different, p < 0.05.

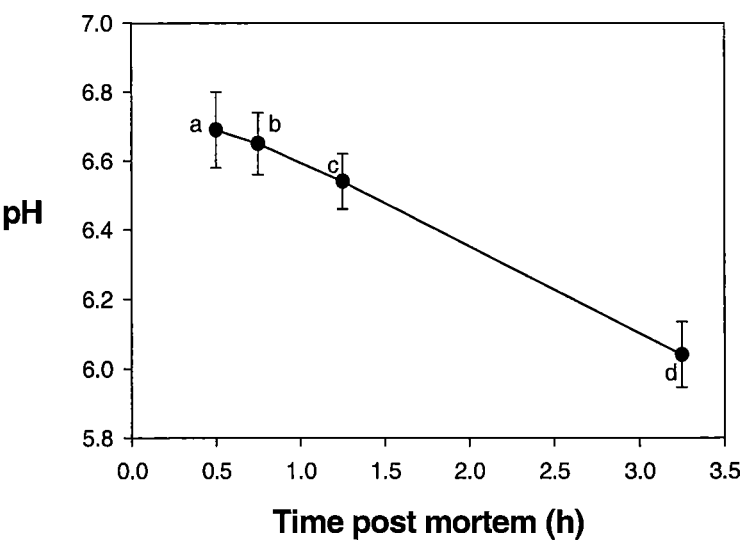


Figure 5.14: Post-mortem pH decline in turkey breast muscle (a = before 1st bath, b = after 1st bath (45 mins PM), c = after 2nd bath (75 mins PM), d = after 1st chill at 0 °C).

The earliest possible time that the pH could be measured in this experiment was 30 minutes post-slaughter and the mean pH at this time was 6.69. During the immersion chilling, the rate of pH decline was 0.0026 units/min whilst in the next two hours the rate of pH decline was 0.0043 units/min (Figure 5.14). This time corresponds with the time that the carcasses

were passed through the second ice slush bath which was maintained at 1.2°C. This period may be crucial in determining the final quality of the meat products.

After the chilling regime, the pH of the muscle from the 'chill + blast' treatment was lower than that of 'chill only' irrespective of whether the muscle was removed at three hours ('off bone'), half breast 'on bone' or both breast muscles left 'on bone'. However, the pH differences were not significant ($p = 0.089$). Interestingly, at six hours post-mortem, the muscle from group B showed significantly lower pH values than group A ('off bone' or 'on bone') ($p = 0.001$).

It is also important to note that the muscles from 'off bone' and 'on bone' were from the same bird and that the mean 'off bone' pH was higher than the 'on bone' pH. Perhaps the most striking feature of this result is that those muscles removed from the carcass at three hours post-mortem ('off bone') and the other half left on the bone ('on bone') had not reached their ultimate pH by six hours post-slaughter. In contrast, those muscles from carcasses that were intact (group B) had reached their ultimate pH at six hours post-mortem. This suggests that leaving the breast muscle 'on bone' for six hours before excision may help to reduce the variability in turkey meat quality caused by pH differences.

5.5.3.2 Turkey breast tenderness

The shear force values of turkey breast main muscle for the various treatments are shown in Table 5.8. The highest shear force was for the 'off bone'/'chill + blast' main muscles. At 24 hours post-mortem, there was no significant difference between the shear force values of muscle from 'off bone'/'chill only' and those from 'off bone'/'chill + blast' although the 'off bone'/'chill only' muscles tended to be lower. At seven days post-mortem, the trends in shear force of the 'off bone' muscle was similar to the trends observed at 24 h post-mortem. However, there were significant differences between 24 h and seven days shear force values of the muscles from 'chilled only' and the 'chill + blast' treatments. The shear force of the 'chilled only' muscles decreased by 26% as a consequence of the 7-day ageing process, whilst the 'chill + blast' muscle shear force values were reduced by 21%. This suggests that the ageing process may be faster in the 'chilled only' muscle compared to the 'chill + blast' muscles. Overall, it appears that even if the muscles were removed from the

carcasses at three hours post-mortem, the tenderness still improved over a 7-day ageing period but not to the same extent as the other treatments.

Table 5.8: Mean + standard deviation for shear force, pH, MFI, and sarcomere length of muscle from ‘off bone’ and ‘on bone’; ‘chill only’ or ‘chill + blast’.

Treatment					
		Breast ‘off bone’ (n = 24)		Breast ‘on bone’ (n = 24)	
Measurement	PM	Chill (n = 12)	Chill+Blast (n = 12)	Chill (n = 12)	Chill+Blast (n = 12)
pH	1	5.93 ± 0.09	5.92 ± 0.08	5.97 ± 0.11	5.91 ± 0.06
	7	5.96 ± 0.08	5.94 ± 0.04	5.87 ± 0.08	5.90 ± 0.05
Shear force (kgF)	1	8.61 ^b ± 1.11	10.3 ^b ± 2.5	4.60 ^a ± 0.49	5.45 ^a ± 0.43
	7	6.39 ^c ± 1.13	8.20 ^c ± 1.78	3.26 ^b ± 0.89	4.35 ^a ± 1.11
Cooking loss (%)	1	19.3 ^b ± 1.61	20.3 ^b ± 1.98	12.1 ^a ± 0.42	12.1 ^a ± 0.32
	7	11.9 ^a ± 0.32	12.0 ^a ± 0.26	11.7 ^a ± 0.45	11.8 ^a ± 0.27
MFI	1	54.8 ^a ± 5.50	51.3 ^a ± 6.80	83.9 ^b ± 10.6	74.0 ^{ab} ± 18.2
	7	100.4 ^c ± 17.7	93.4 ^c ± 18.9	117.8 ^c ± 14.3	112.2 ^c ± 10.5
Sarcomere length (µm)	1	1.69 ^a ± 0.20	1.70 ^a ± 0.18	2.05 ^b ± 0.14	1.92 ^b ± 0.11
	7	1.77 ^a ± 0.14	1.73 ^a ± 0.09	1.96 ^b ± 0.07	1.89 ^b ± 0.06

^{abc} Means in the same row with different superscript are significantly different, p< 0.05, PM = post mortem time

The ‘on bone’ muscle showed a similar shear force trend as the ‘off bone’ muscle at day 1 post-mortem in that at 24 h post-slaughter the average shear force of the ‘on bone’ muscle that was treated with ‘chill + blast’ was 16% higher than ‘chill only’ muscle. After seven days of ageing, the ‘chill + blast’ muscle mean shear force was significantly higher than that of ‘chill only’ muscle. Again, ‘chill only’ muscle had significantly lower shear force values at seven days post-mortem compared to 1-day muscle (p= 0.025). However, seven days of ageing had no significant effect on the shear force of muscle that were subjected to the ‘chill + blast’ treatment. The shear force of the ‘chill only’ muscle decreased by 29% and the ‘chill + blast’ muscle shear forces by 20% over the seven days post-mortem period. Based on the above observations, it seems tenderness improvement occurred more in the ‘chill only’ muscles ‘on bone’ than ‘off bone’ muscles over the ageing period. Muscles derived from ‘on bone’ or ‘off bone’ using chill and blast had similar rates of ageing.

Table 5.9: Pooled mean + standard dev. for shear force, pH, MFI, cooking loss and sarcomere length of muscle ‘off bone’, ‘on bone’ and ‘aged on bone for seven days’

Treatment	PM	pH	Shear force (kgF)	Cooking Loss (%)	MFI	Sarcomere length (μm)
Breast ‘off bone’ (n=24)	1	5.93 ^a ± 0.09	9.47 ^a ± 3.83	19.8 ^a ± 1.84	53.0 ^a ± 11.0	1.69 ^a ± 0.18
	7	5.95 ^a ± 0.07	7.29 ^b ± 2.66	12.0 ^b ± 0.29	96.9 ^c ± 18.2	1.75 ^a ± 0.11
Breast ‘on bone’ (n=24)	1	5.94 ^a ± 0.09	5.02 ^c ± 1.56	12.1 ^b ± 0.40	78.9 ^b ± 19.7	1.99 ^b ± 0.14
	7	5.88 ^b ± 0.05	3.80 ^d ± 1.21	11.8 ^b ± 0.36	115 ^d ± 12.6	1.93 ^b ± 0.07
Aged on Bone (n=24)	7	5.88 ^b ± 0.05	3.23 ^d ± 0.36	12.3 ^b ± 0.27	124.5 ^e ± 7.6	2.01 ^b ± 0.09

^{abcde} Means in the same column with different superscript are significantly different, p< 0.01, PM= post-mortem, MFI = myofibrillar fragmentation index

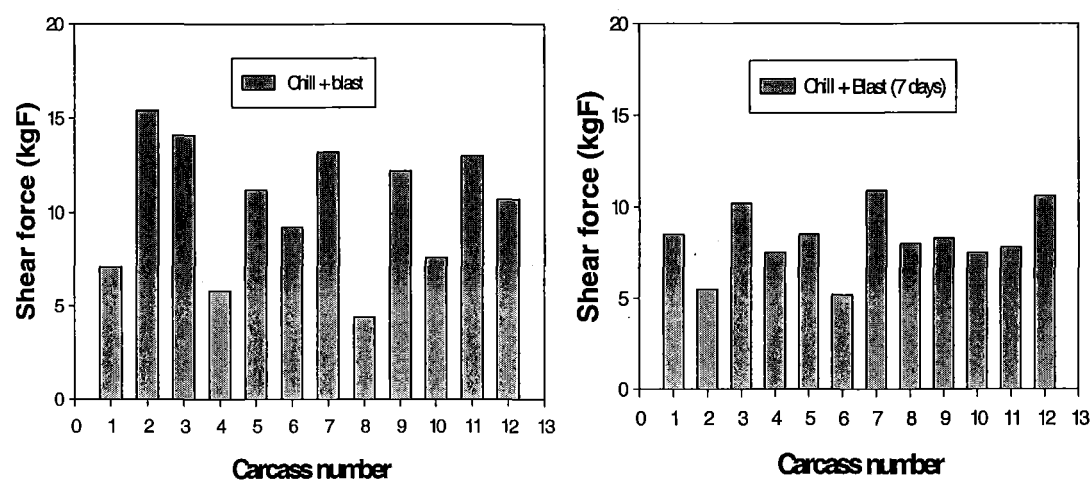
Table 5.9 shows the pooled mean for shear force, MFI, cooking loss and sarcomere length of the breast muscle removed three hours post-mortem (‘off bone’) and the breast left on the bone (‘on bone’) from the same bird. At 24 hours post-mortem, the muscle taken off the bone at three hours post-mortem was significantly tougher than the opposite muscle left to age on the bone. The shear force of ‘off bone’ muscle was almost double the ‘on bone’ at 24 hours post-mortem (Table 5.9). After seven days of storage, the shear forces of the ‘on bone’ muscle was significantly lower than the corresponding ‘off bone’ muscle. It is interesting to note that the ‘on bone’ muscle was more tender at 24 hours post-mortem than the corresponding ‘off the bone’ muscle aged seven days.

In both the ‘off bone’ and ‘on bone’ muscle from the same carcass, there were significant differences in the shear forces at 24 h and seven days post-mortem. The result indicates that at seven days post-mortem, the muscles in both cases were more tender than the 24 h post-mortem muscles although at both times the ‘on bone’ muscle was more tender than the ‘off bone’ muscle. Furthermore, there was a wider variation in shear forces of ‘off bone’ muscle than the ‘on bone’ muscle (Figure 5.15).

The muscles that remain intact on the bone throughout the seven days of ageing produced superior tenderness results. Their mean shear force value was lower than muscles from ‘on the bone’ at 24 h post-slaughter (Table 5.9). However, the differences in the shear force values were not significant. It appears that leaving the muscle to age on bone for seven days

improves tenderness. The results for cooking loss are shown in Table 5.5. There was no evidence that ‘chill only’ or ‘chill + blast’ affects the cooking loss of turkey breast muscle. However, the cooking losses of muscle from ‘off bone’ (either ‘chill only’ or ‘chill + blast’) were significantly lower at seven days compared to the 24 h post-mortem muscles. In contrast, the cooking loss of muscle from ‘on bone’ was similar at all post-mortem times irrespective of the various treatments. At 24 h post-slaughter, the cooking loss of muscles from ‘on bone’ was significantly lower than from ‘off bone’ muscles even though the muscles are from the same turkey.

Breast muscle ‘off bone’ at three hours post-slaughter



Breast muscle ‘on bone’ for 24 hours

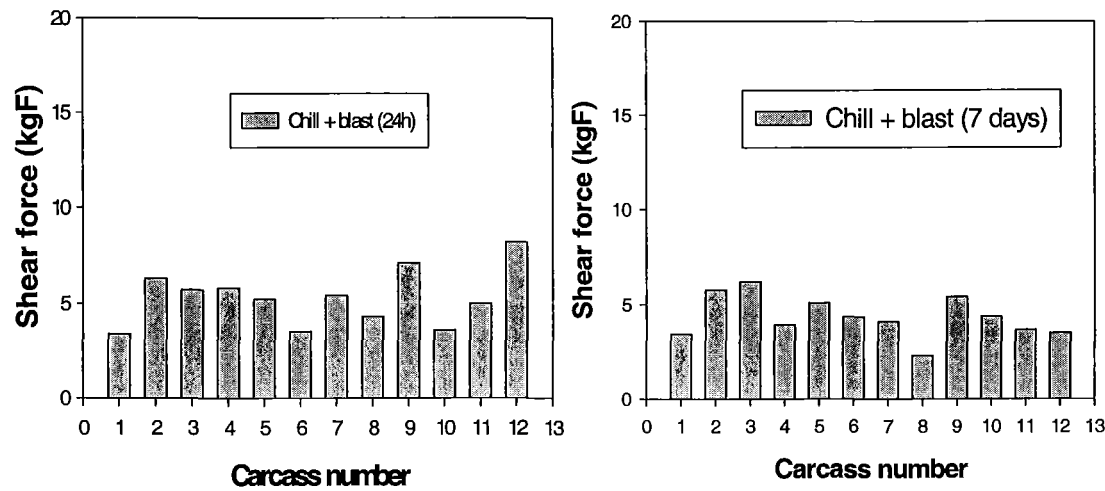


Figure 5.15: Existing (‘off bone’) and new (‘on bone’) processing methods.

5.5.3.3 Myofibrillar fragmentation index (MFI) and Sarcomere length

A high MFI value indicates increased myofibril fragmentation due to proteolysis. In this study, at 24 hours and seven days, there was no significant differences between the MFI of muscle 'off bone' /'chill only' and 'off bone'/'chill + blast' although muscles from 'chill + blast' tended to have lower MFI than 'chill only' muscles. The effect of the treatments on the MFI of muscle from 'on bone' (chill only' or chill + blast) was similar to the results for the 'off bone' muscles (Table 5.8). However, the MFI of 'on bone' muscles was higher than the MFI from 'off bone' muscles irrespective of the treatments and ageing period. At day 1 or day 7 post-slaughter, the MFI of 'off bone' muscles was significantly lower than 'on bone' muscles (Table 5.9). Despite the muscles originating from the same turkey, removing the muscles at three hours post-mortem significantly reduced the extent of myofibrillar protein degradation post-mortem. Moreover, the MFI value was significantly higher in muscles aged on the bone for seven days compared to those removed from the bone at three hours or 24 hours and then aged for a total of seven days. The implication of this is that leaving the muscle 'on bone' enhances proteolysis and consequently improves meat tenderness.

The extent of muscle contraction was reflected and measured by the length of the sarcomeres. There was no evidence to indicate that 'chill only' or 'chill + blast' had any effect on the sarcomere length of the muscle fibres from the 'off bone' and 'on bone' muscles (Table 5.8). More importantly, the sarcomere length of 'off bone' muscles were shorter than those of 'on bone' muscles (Figure 5.16). This means that the muscles removed three hours post-slaughter experienced muscle shortening, whilst those left to age on the bone did not shorten. In addition, unlike experiment 1, where the sarcomere lengths were shorter during ageing, in this case the sarcomere lengths of muscle deboned three hours post-slaughter tended to increase with ageing.

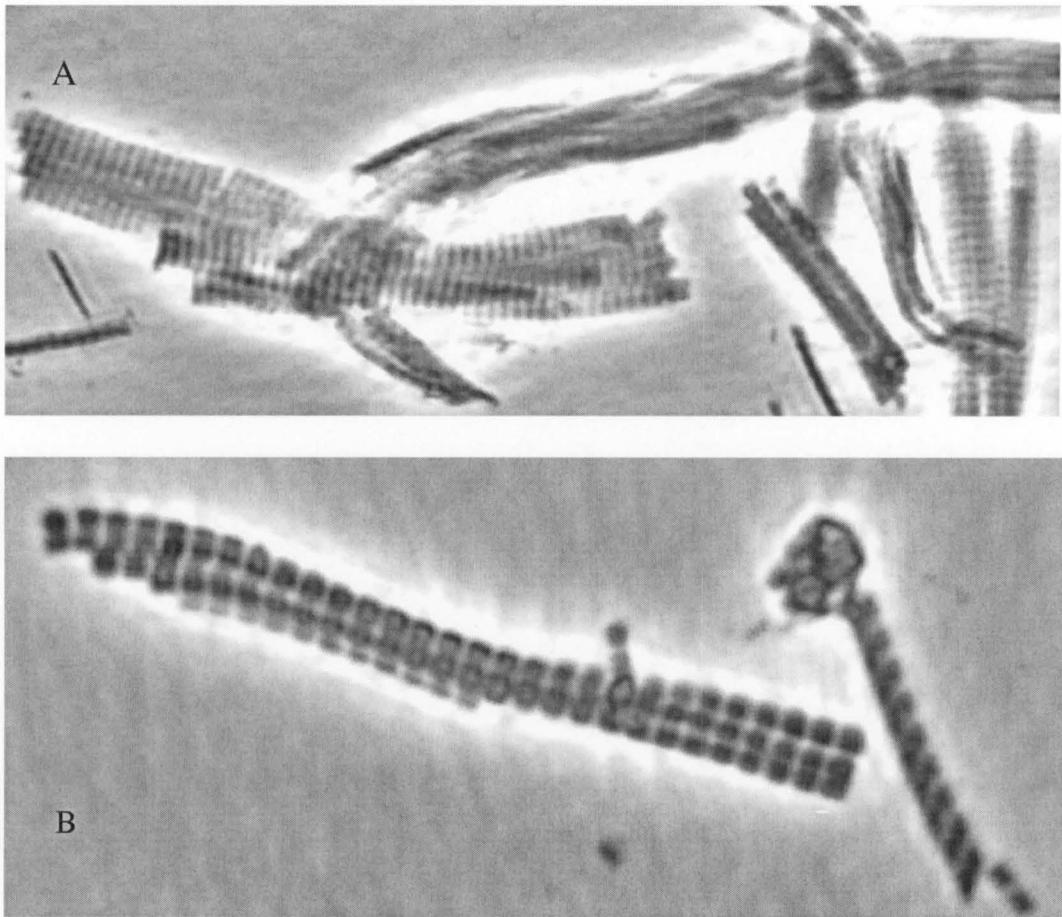


Figure 5.16: Phase contrast micrograph of myofibrils at 24h post-mortem (Magnification x 630), A is from turkey breast muscle deboned 3 h post-slaughter and B is from muscle deboned 24 h post-slaughter.

5.5.3.4 Relationship between ultimate pH, shear force, MFI and sarcomere length

The relationship between the ultimate pH and shear force for ‘off bone’ and ‘on bone’ muscles at 24 hours are shown in Figure 5.17. There was a negative relationship between the pH of ‘on bone’ or ‘off bone’ muscles and the shear force at 24 hours post-mortem indicating that meat of high pH had lower shear force values ($r = -0.488$ and -0.405 , respectively). However, there was no correlation between pH and the shear force of ‘off bone’ or ‘on bone’ muscles at seven days post-slaughter. It is obvious in Figure 5.17 that the ‘off bone’ muscles were tougher than the ‘on bone’ muscle. In both ‘off bone’ and ‘on bone’ muscles, as the ultimate pH increased from about 5.80 to 6.25, the turkey breast meat became more tender after ageing for 24 hours.

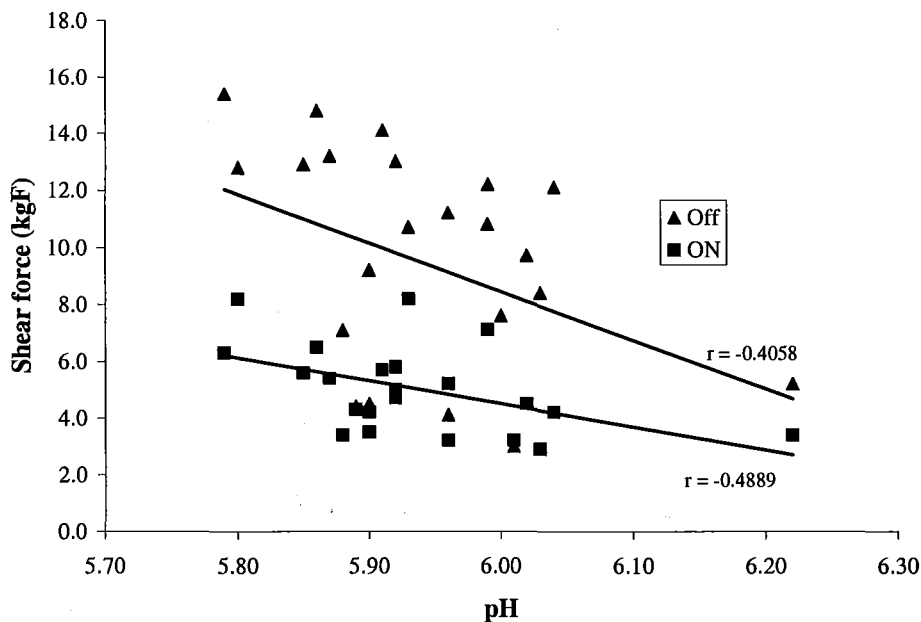


Figure 5.17: The relationship between ultimate pH and shear force of muscles from 'on bone' and 'off bone' at 24 h post-mortem.

The effect of pH on the degradation of myofibrils mirrors the effect of pH on the shear force values. At 24 hours post-mortem, the degradation of the myofibrils increased as pH increased for the 'on bone' ($r = 0.652$, $p = 0.01$) and 'off bone' ($r = 0.449$, $p = 0.01$) muscles. Clearly, the degradation of myofibrils was more related to ultimate pH in the 'on bone' muscle compared to the 'off bone' muscle (Figure 5.18). Also, after seven days of ageing, the pH was positively correlated with the MFI of muscles from 'on bone' ($r = 0.492$) but not for the 'off bone' muscles.

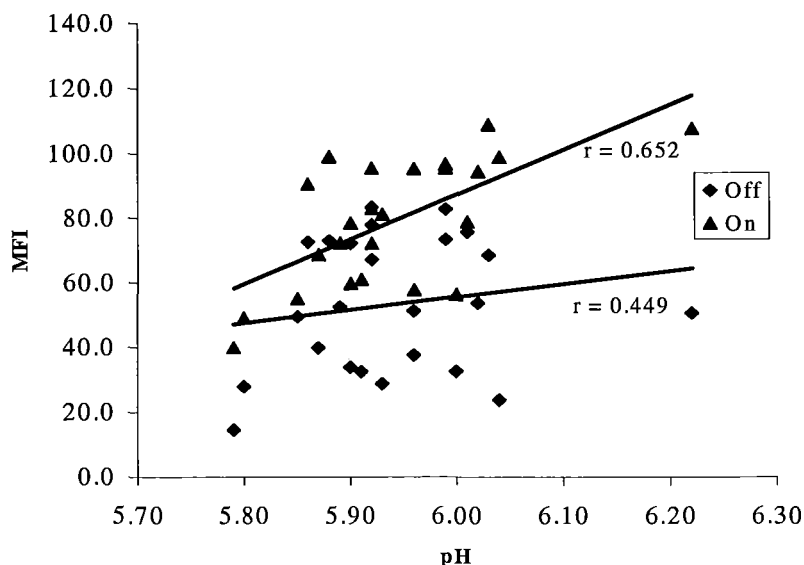


Figure 5.18: The relationship between ultimate pH and MFI of muscles from 'on bone' and 'off bone' at 24 h post-mortem.

At 24 hours post-mortem, the sarcomere length was negatively correlated with the shear force of 'off bone' muscles ($r = -0.680$, $p = 0.01$) (Figure 5.19). This suggests that the toughness of meat from 'off bone' muscles may be a result of muscle contraction. On the other hand, there is no evidence to suggest that sarcomere length had any effect on the tenderness of muscle aged on the bone.

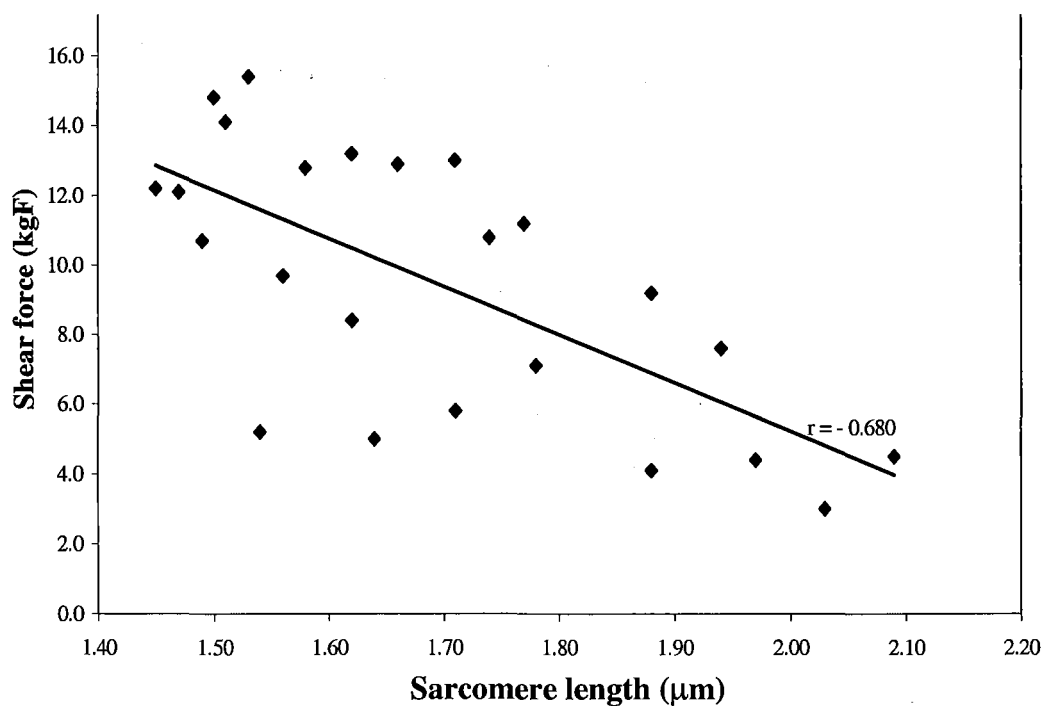


Figure 5.19: The relationship between sarcomere length and shear force for muscle 'off bone' at 24 h post-mortem.

5.5.3.5 Electrophoresis

Electrophoretic analysis was performed on all samples. However, for presentation of the results, representative SDS-PAGE profiles of myofibrillar protein extracts from 'off bone' and 'on bone' muscles are shown in Figures 5.20a and 5.21a respectively. There was no obvious difference between the banding patterns of the SDS-PAGE profiles of myofibrillar proteins extracted from 'off bone' and 'on bone' muscles at 1-day and 7-day post-mortem. This may imply that the rate of ageing as a result of proteolysis is similar in both 'off bone' and 'on bone' muscles.

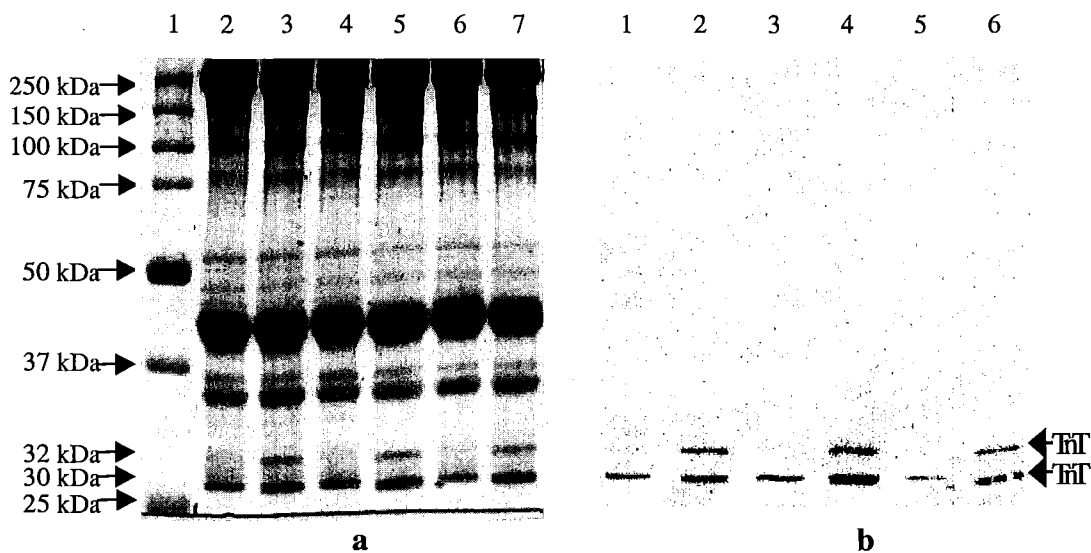


Figure 5.20(a): Coomassie brilliant blue stained 12.5% gel of SDS-PAGE and (b) western blotting analysis of turkey breast muscle (deboned 3 h post-slaughter) proteins from myofibrillar fraction extract at 24h and 7 days of ageing. Each lane was loaded with 20 μ g of protein in the extract. In 'a' Lane 1 = MWM; lanes 2,4,6 = 1 day, lanes 3,5,7 = 7 days post-mortem samples; and in 'b' lanes 1,3,5 = 1 day, lanes 2,4,6 = 7 days post-mortem samples.

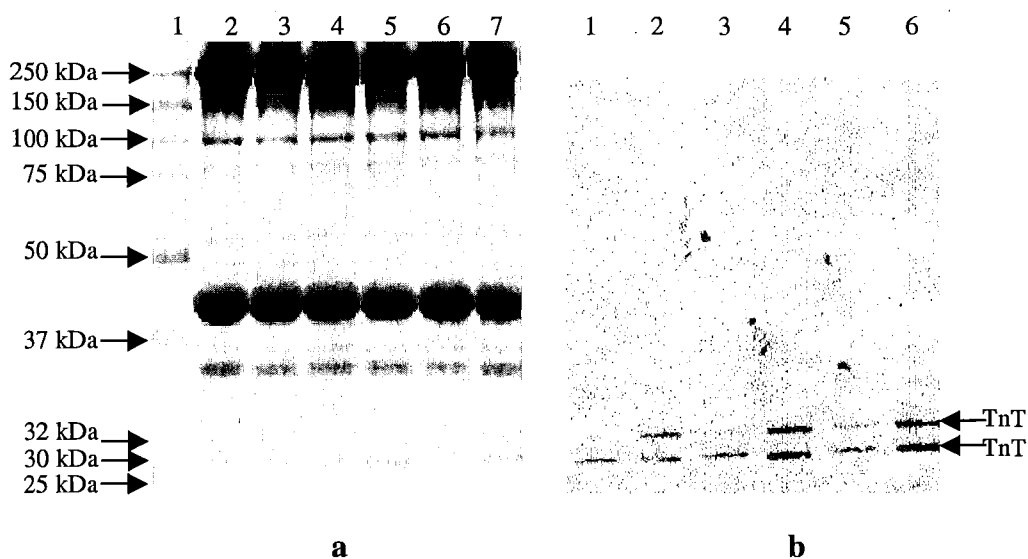


Figure 5.21(a): Coomassie brilliant blue stained 12.5% gel of SDS-PAGE and (b) western blotting analysis of turkey breast muscle (aged on bone) proteins from myofibrillar fraction extract at 24h and 7 days of ageing. Each lane was loaded with 20 μ g of protein in the extract. In 'a' Lane 1 = MWM; lanes 2,4,6 = 1 day, lanes 3,5,7 = 7 days post-mortem samples; and in 'b' lanes 1,3,5 = 1 day, lanes 2,4,6 = 7 days post-mortem samples.

The myofibrillar protein profiles revealed that 30 and 32kDa fragments began to appear at 1-day post-mortem in both 'off bone' and 'on bone' muscles, although the 32kDa fragments was more obvious at seven days compared to 1-day. Western blotting was performed on the samples using anti-troponin T. The 30kDa and 32kDa polypeptides were

labelled by anti-troponin T, indicating that they are the degradation of troponin T (Figure 5.20b and 5.21b).

5.5.4 Conclusion

It is evident in this experiment that the deboning of turkey breast muscle three hours post-slaughter toughened the meat. Ageing the breast muscle on bone for seven days significantly improves meat tenderness. In addition, turkey breast muscles that are deboned three hours post-slaughter or 24 hours post-mortem and then aged for a further six days may achieve an acceptable tenderness. The higher shear force values at 24 hours of the muscles deboned three hours post-slaughter was a result of cold shortening as evidenced by the shorter sarcomere length of these muscles compared to muscles aged on bone for 24 hours before deboning. Also, the significantly higher shear force values of muscle deboned three hours post-slaughter may be due to reduced proteolysis compared to muscles aged on the bone for 24 hours before deboning. Finally, the results of this experiment confirmed experiment 2 that applying a cold blast to turkey breast muscle has no advantage over a chill only treatment in terms of meat tenderness.

5.6 Discussion

5.6.1 Post-mortem temperature and pH decline in turkey breast muscle

The rate of post-mortem temperature decline in the turkey breast muscles showed a similar trend in all the experiments. The carcass temperature ranged from 32 to 36°C at 15 minutes post-slaughter. This was lower than previous reports for turkey breast muscle. Wynveen et al (1999) reported a mean internal breast muscle temperature of 42.4°C at 14 minutes post-slaughter and Pietrzak et al (1997) reported a range of 41-44°C. In both of these experiments, the birds were scalded for 2.5 minutes at 70°C. In this work, the birds were scalded for 45 seconds at 63°C followed by a 60 second shower cooling. This probably explains the lower inner breast muscle temperature observed in this work.

As expected the immersion chilling (1st water bath and 2nd ice slush bath) rapidly reduced the internal breast muscle temperature to about 10°C by 75 min after stunning. This is consistent with a previous study in turkey, which showed a sharp decline in internal breast

muscle temperatures due to chilling in water bath (Wynveen et al, 1999). Although there was a sharp decline in the internal muscle temperature after the immersion chilling, the rate of temperature decline differs in the three experiments; 0.35°C/min in experiment 2, 0.55°C/min in experiment 3 and 0.43°C/min in experiment 4. The differences in the rate of cooling may be attributed to the variation in the weight of the turkeys used in each of the experiments.

In all three experiments (2, 3 & 4), cold blast treatment significantly decreased the inner breast muscle temperature compared to the breast muscle that was chilled only. The breast muscle temperature immediately after a cold blast treatment (about six hours post-slaughter) ranged from 2-6°C irrespective of whether the muscles were deboned or left on bone. This chilling regime is designed to improve the shelf life of turkey breast meat by lowering carcass temperature to about 4°C within 6 hours post-slaughter. The low temperature would reduce bacterial spoilage associated with high pH meat.

The post-slaughter pH decline in turkey breast muscle is shown in Figures 5.6, 5.10 & 5.14. In this study, the pH of turkey breast muscle at 15 min post-slaughter ranged between 6.1-6.94. Northcutt et al (1998a) observed that the mean muscle pH at 15 min post-slaughter of electrically stunned 18-wk old turkey was 6.36. This finding is similar to the mean pH of 6.4 observed in the present study. The rate of pH decline in the turkey breast muscle was most rapid for the first 15 min post-slaughter. This result is consistent with previous studies which showed that the breast muscle of turkey exhibit accelerated rigor mortis compared to beef or lamb (Sosnicki and Wilson, 1991; Wynveen et al, 1999; Sosnicki, 1993).

The pH of the breast muscle from double electrically stunned carcasses was significantly lower than the pH of muscle from single stunned carcasses at 15 minutes through to 60 minutes post-slaughter. The lower breast muscle pH of double stunned carcasses indicates a faster rate of glycolysis in these muscles compared to muscles from single stunned carcasses. This suggests that the delay in rigor mortis development may not be due to the direct effect of electrical stunning on post-slaughter muscle metabolism. Rather, it may be a result of reduced struggling of the birds (Papinaho et al, 1995; Murphy et al, 1988). The duration of electrical stunning may be important in that prolonged electrical stunning may accelerate rigor mortis which will impact on the overall meat quality.

In all three experiments (2, 3 & 4), the pH of turkey breast muscle at six hours post-slaughter was close to the ultimate pH at 24 hours. The mean ultimate pH (pH_u) of turkey breast muscles ranged from 5.80 to 5.96. In experiment 1, the pH at seven days post-mortem was significantly lower than at one-day post-mortem (Table 5.1). In contrast, in experiment 2 the pH tended to be higher at seven days than at 1-day post-slaughter in experiment 2. Wakefield et al (1989) observed a similar increase in chicken muscles pH aged four days, although it has not been reported in turkey muscles. The increase in pH of turkey muscle aged for seven days in this work also occurs in beef. It is thought to be a consequence of the denaturation and proteolysis of muscle proteins (Wierbicki, et al, 1954).

5.6.2 Effects of chilling on the tenderness of turkey breast meat

Muscles from turkeys undergo rapid post-mortem glycolysis and, as a consequence, may exhibit many of the same biochemical and quality defects as occur in PSE pork. In order to avoid this quality defect in turkey breast meat, processors employ a rapid chilling procedure which involves the immersion of turkey carcasses into cold water and ice-slush baths at temperatures of 4°C and 1°C respectively. However, placing freshly-slaughtered and eviscerated carcasses into chill baths of near 0°C may alter post-mortem changes in meat and, consequently, affect the tenderness of the end product (Welbourn et al, 1968). It has been shown by several researchers that low immersion chilling temperatures induce cold shortening of chicken muscles (Papa and Fletcher, 1988; Smith et al, 1969; Lee and Rickansrud, 1978).

The effect of rapid cooling by ice-slush baths on the tenderness of turkey breast meat was investigated in experiment 2. The results showed that muscles assessed at 24 hours post-mortem from carcasses passed through the ice-slush twice were as tender as those muscles from carcasses bathed once. However, after ageing the muscles for six days, the 'bath once' muscle was more tender than the 'bath twice' muscle. This was independent of whether the muscle was deboned three hours post-slaughter or aged 'on bone' for 24 hours. It was assumed that passing the carcasses through the ice slush bath a second time would decrease the muscle temperature and slow down the rate of glycolysis even more and, consequently, affect the characteristics of the muscle. However, the extent of muscle contraction was

similar in the 'bath once' muscle and 'bath twice' muscle since the sarcomere lengths were similar in both cases.

It appears that the decline in inner muscle temperature was sufficient after passing through the ice slush bath once, since any further reductions in temperature from a second wash had no significant effect on meat tenderness. The slight differences in the tenderness of the 'bath once' muscle and 'bath twice' muscle at seven days post-mortem cannot be explained by any differences in the extent of muscle contraction known as cold shortening. The slight differences in the tenderness of 'bath once' muscle and 'bath twice' muscle may be due to the effect of the rapid chilling on the enzyme activities such as calpains which have been implicated in post-mortem tenderisation. The effect of the cold blast (-24°C) treatment on turkey breast meat was also investigated. The tenderness of turkey breast muscle was not affected significantly by the cold blast treatment.

5.6.3 Effect of 3 h post-slaughter deboning on the tenderness of turkey breast meat

One of the objectives of this work was to investigate the effects of deboning turkey breast muscle at three hours post-slaughter on the overall tenderness of the meat. After-slaughter, rigor development occurs which involves the gradual shift of muscle cell metabolism from aerobic to anaerobic pathways in which ATP is depleted and actomyosin is formed (Hamm, 1982). This rigor mortis process requires four hours to occur in broiler breast muscles and, if meat is deboned prior to this time, then the meat will toughen (Dawson et al, 1987). To my knowledge, the time for rigor mortis to occur in turkey breast muscle has not yet been defined.

The results of this research indicate that muscles aged 'on bone' for 24 hours prior to deboning were very tender compared to turkey breast muscles deboned at three hours post-slaughter in experiments 3 & 4. In experiment 3, all the muscles were aged 'on the bone' throughout the ageing period and all the meat was very tender. These results do not support those reported by Alvarado and Sams (2000) who suggested that deboning turkey breast at two hours post-mortem or later will not significantly impair meat tenderness.

In this work, turkey breast muscles deboned at three hours post-slaughter were tougher than the breast muscles aged on the bone for 24 h, due to muscle shortening. The extent of cold shortening of the muscle deboned three hours post-slaughter compared to muscles deboned 24 hours post-mortem is shown in Figure 5.16. In this figure, the muscles were harvested from the same turkey and at the same location. Muscle sample 'A' was from the left breast muscle deboned at three hours post-slaughter, whilst muscle sample 'B' was from the right muscle aged 'on the bone' for 24 hours. Clearly, the muscle deboned at three hours post-slaughter experienced severe shortening. The muscles left intact 'on the bone' for 24 hours before deboning were relaxed. Furthermore, complete rigor mortis development and ageing took place in the breast muscle aged 'on the bone' for 24 h before deboning. No meat toughening occurred due to the premature removal of the turkey breast muscle. Since muscles 'A' and 'B' went through both cold baths, the toughening of the turkey breast muscle excised three hours post-slaughter is not due to the effect of the ice-slush bath (1°C) but to the exposure of the deboned muscle to the chilling regime.

5.6.4 Effects of ageing on the tenderness of turkey breast meat

The effect of extended ageing on the tenderness of turkey breast meat was also evaluated in this study. It is known that post-mortem ageing of muscles improves red meat tenderness (Goll et al, 1992; Koohmaraie, 1992, Geesink and Koohmaraie, 1999a). There is, however, insufficient data in the literature which describes the effects of extended ageing on the tenderness of turkey breast meat. However, numerous studies have evaluated the tenderness of chicken fillets deboned pre-rigor and subsequently aged for 20-72 hours post deboning (McKee et al, 1997; Smith et al, 1992; Sams et al, 1990; Lyon et al, 1992). Lyon et al (1992) observed that increasing the ageing time after pre-rigor boning had no significant effects on meat tenderness. In contrast, Hirschler and Sams (1994) observed a significant decrease in shear force values between 24 and 72 hours of post excision refrigerated ageing. In another study, McKee et al (1997) demonstrated that although there was no improvement in the tenderness of fillets due to extending post-slaughter deboning and ageing from 0 to 23 hours, they observed improved tenderness if ageing was extended up to 72 hours post-mortem.

In the preliminary experiment in this investigation (experiment 1), turkey breast muscle deboned at three hours post-slaughter toughened between one and seven days of ageing. In addition, there was a significant decrease in the pH of turkey breast main muscle between 24 hours and seven days post-mortem. The unusual toughening of turkey breast muscles over the seven days ageing period is difficult to explain. However, a possible reason for the observation may be that the breast muscles used to determine the shear force values at each post mortem times originated from different turkeys. It may also be due to an incorrect chilling regime and electrical stunning applications which produced the inappropriate pH and temperature declines post-slaughter. Consequently, the muscle may have experienced cold shortening over the extended ageing period.

In experiment 2, although the meat did not toughen with ageing, the results were similar to what other researchers have observed for broiler breast meat (Lyon et al, 1992). There was no improvement in the tenderness of turkey breast meat deboned at three hours and 24 hours post-slaughter and aged for six days. In contrast, experiment 4 clearly showed that extending the ageing of pre-rigor or post-rigor deboned turkey breast muscle significantly improved meat tenderness (Table 5.9). In both muscles deboned at 3 h and 24 h post-slaughter, there was a 25% decrease in the shear force values after the 6-day ageing period. The implication of these observations is that processors who debone turkey breast muscle immediately after chilling may produce meat with acceptable tenderness values if they ensure that the muscles are sufficiently aged.

To elucidate the mechanisms involved in the observed shear force differences in turkey breast muscle deboned at three or 24 hours post-slaughter and the observed improvement in tenderness over the extended post-mortem period, the relevant muscle biochemical and physical parameters were measured. For example, sarcomere lengths of breast muscles were determined as it has been reported that there is a relationship between sarcomere length and meat tenderness (Herring et al, 1967; Owens and Sams, 1997). Other reports have indicated there is no relationship (Smulders et al, 1990 and DeVol et al, 1988). In chicken, harvesting *Pectoralis major* muscles pre-rigor stimulates the muscles and, as a consequence, sarcomeres shorten due to the loss of skeletal restraint (Sams et al, 1990).

Experiment 4 shows that the sarcomere lengths of muscles deboned three hours post-slaughter were significantly shorter than the sarcomere lengths of muscles deboned at 24

hours post-slaughter (Table 5.9). Furthermore, all the muscles that were aged on the bone for 24 hours prior to deboning had longer sarcomere lengths. Consequently, the meat was very tender (Figure 5.11). The differences in the sarcomere lengths of 'off bone' muscle and 'on bone' muscle is likely to be due to treatment effects rather than sampling effects because each breast muscle sample was taken from the middle of the breast muscle from the same bird.

Figure 5.19 shows the relationship between sarcomere length and 24 h shear force for muscle deboned three hours post-slaughter. Muscles with long sarcomere lengths had lower shear force values than muscles with short sarcomeres. Marsh and Leet (1966) reported a similar result in beef. The results reported in this work suggest that shortened sarcomeres in muscles deboned three hours post-slaughter are responsible, in part, for the observed toughness of turkey breast meat. The shortening of the sarcomere length is because rigor mortis was not completed before muscle excision. Hence, the muscles experienced cold shortening due to the loss of skeletal restraint (Sams et al, 1990). However, the corresponding muscles left on the bone for 24 hours before deboning did not cold-shorten due to the restraint provided by the skeletal framework.

Rigor development should be complete by 24 hours post-slaughter and the resolution of rigor-locked sarcomeres should result in improved meat tenderness (Lawrie, 1991). It was observed in experiment 2, that the sarcomere lengths of 'off bone' muscle and 'on bone' muscle in all the treatments decreased by between 7-9% after seven days of ageing. Although this decrease in sarcomere lengths may account for the tendency of the shear force values of the muscles to increase, the significantly shorter sarcomere lengths observed during the extended ageing of turkey breast muscle are difficult to explain. It is known that if muscles containing high levels of ATP are subjected to a very low temperature they will shorten (Bendall et al, 1976). In other words, after the death of an animal/bird, if all the ATP is not depleted during rigor mortis development before freezing the carcasses, then the resulting meat will toughen.

However, in experiment 4 the sarcomere lengths of the muscle fibres only changed slightly during six days of extended ageing irrespective of whether the muscles were excised three hours post-mortem or deboned 24 hours. The insignificant changes in sarcomere lengths of the turkey breast muscle cannot explain the significant improvement in the tenderness of

the muscles originating from carcasses deboned at three hours post-slaughter and 24 hours post-mortem, respectively. Thus, the improvement in the tenderness over the 6-day extended ageing period must be due to other mechanisms involved in the post-mortem tenderisation such as proteolysis (Koohmaraie, 1992) or ionic strength differences (Ouali, 1992).

The myofibrillar fragmentation index (MFI), which is a measure of proteolysis, is directly related to the degree of fragmentation of myofibrils. High MFI values indicate increased proteolysis due to muscle proteases (Geesink and Koohmaraie, 1999a). In experiment 2, the extent of proteolysis, as measured by the MFI at seven days, was not significantly different from the MFI at one day post-mortem. This is different from the report on lamb LD (Geesink and Koohmaraie 1999a) which showed that MFI increased during post-mortem ageing of lamb LD. The observed MFI values indicate that the tenderising effects of proteolysis were unable to overcome the toughening effects of muscle contraction as indicated by the shortening of the sarcomeres.

Another possible explanation is that the shortening of the sarcomeres during the six day ageing period, may reduce the filament surface area and, consequently, the accessibility of proteolytic enzymes to muscle proteins. In contrast, experiment 4 suggested that the MFI was significantly higher at seven days of ageing compared to one-day post-slaughter for both muscles deboned at 3 h and 24 h post-slaughter. Therefore, myofibrillar proteins degradation (proteolysis) may, in part, be responsible for the reduced shear force values of turkey breast meat during post-mortem ageing in this experiment. Similar results have been reported in chicken (Birkhold and Sams, 1992) and lamb (Koohmaraie, 1994) muscles. The increase in fragility of the myofibrils may be due to the weakening or degradation of desmin as reported by Geesink and Koohmaraie (1999b).

The disappearance of troponin T is accompanied by the appearance of 28-32 kDa polypeptides (Koohmaraie, 1996; O' Halloran, 1997). In this research, SDS-PAGE analysis of the myofibrillar proteins of muscles aged 'on the bone' and 'off the bone' showed that the 30 kDa polypeptide began to appear at 1-day post-slaughter. However, the presence of the 32 kDa polypeptide was only obvious at seven days post-mortem in both muscles. This observation shows that there was more proteolysis in the muscles aged for seven days than at one day post-mortem and, as a consequence, improved meat tenderness over the 6-day

ageing period. In addition, because the banding pattern of the myofibrillar proteins (Figure 5.20a & 5.21a) is similar for both 'off the bone' and 'on the bone' muscles, it may be concluded that the extent of proteolysis in both muscles were equivalent. Therefore, the higher shear force values at 24 h of muscles deboned three hours post-slaughter compared to muscles aged on bone for 24 h, may be due largely to shorter sarcomere length and not to reduced proteolysis. The 30 and 32 kDa polypeptides were probed with anti-troponin T (Figure 5.20a & 5.21a). Clearly, the polypeptides were identified as troponin T degradation products which is consistent with the report of Ho et al (1994) who demonstrated that the polypeptide 30 kDa is a product of troponin T degradation.

The relationship between ultimate muscle pH and meat tenderness has been studied extensively in beef (Purchas and Aungsupakorn, 1993) and lamb (Watanabe et al, 1996). Correlation analysis of the data from experiment 4 showed there was a significant relationship between ultimate pH (pH_u), shear force and MFI of both turkey breast muscles deboned at three hours post-slaughter and the corresponding muscles deboned at 24 h post-mortem. Shear force and pH_u had an overall correlation coefficient of -0.405 and -0.488 for muscles deboned at three hours and 24 hours post-mortem, respectively (Figure 5.17). This relationship indicates that cooked turkey breast meat was less tender if the pH_u was low. This trend is consistent with the results of other researchers, who have also shown a negative relationship between pH_u and shear force in meat from chicken (Stewart et al, 1984).

A curvilinear relationship between pH_u and shear force has been observed in beef (Purchas, 1990; Obanor et al, 2001) and lamb (Watanabe et al, 1996). These researchers reported that muscles with pH_u between 5.8 and 6.2 had the highest shear force values, whilst those with pH_u values below or above this range had lower shear force. The negative relationship between pH_u and shear force of turkey breast muscle in this work is similar to those findings since the range of pH_u was between 5.8 and 6.27. Furthermore, the pH_u was positively related to MFI at 24 hours post-mortem for muscles deboned at three hours and at 24 hours post-slaughter. This indicates that as the pH_u increased from 5.8 to about 6.30, the myofibrils tend to be more fragile. As discussed earlier, high pH_u turkey breast meat tends to be more tender than low pH_u breast meat. Thus, increased tenderness is associated with increased fragmentation of turkey muscle myofibrils. Similarly, Watanabe et al. (1996) found that lamb LD muscle with high pH_u (> 6.1) was more tender and had a high

MFI. MFI is a measure of the fragmentation of myofibrillar protein. The increase in MFI as the pH_u increases in turkey breast muscle may indicate increased proteolysis by muscle proteases, such as calpains. The activity of calpains is maximal at around neutral pH.

Shear force was also negatively correlated with the sarcomere length of muscles deboned at three hours post-slaughter but there was no relationship between the shear forces of muscles deboned at 24 hours post-mortem and sarcomere length. Thus, with increasing sarcomere lengths, the meat from carcasses deboned 3 h post-slaughter had a tendency to become more tender on ageing. Based on these relationships, it appears that tougher muscles have lower pH values than the more tender muscles. It may be suggested that this indirect relationship arises because pH_u is related to the time of onset of rigor mortis, the rate of cooling and the extent of myofibrillar contraction.

Chapter 6

Changes in calpain I, II, and calpastatin in turkey breast muscle during post-mortem ageing

6.0 Introduction

Deboning of turkey breast muscle three hours post-slaughter in section 5.5 toughened the resulting meat. The higher shear force at 24 hours of the muscles deboned three hours post-slaughter was a result of muscle contraction, as evident by the shorter sarcomere lengths of these muscles compared to muscles aged on bone for 24 hours prior to deboning. In addition, it was observed that turkey breast muscle deboned three hours post-slaughter had lower myofibrillar fragmentation index (MFI) values compared to muscles deboned at 24 hours. MFI is a measure of the extent of proteolysis with higher MFI values indicating increased proteolysis. The lower MFI values of muscles deboned three hours post-slaughter may be due to reduced activation of the calpains. Alternatively, it may be that the calpain is less effective because of inaccessibility to the myofibrillar proteins as a consequence of the shorter sarcomere lengths of the muscles. In order to resolve this issue, the activity of the calpain proteolytic system (calpain I, II and calpastatin) was determined in the muscles deboned at three hours and compared with the activity in muscles deboned at 24 hours post-slaughter.

Proteolysis of the myofibrillar proteins by the calpain proteolytic system is widely accepted to be responsible for meat tenderisation during post-mortem ageing (see section 2.4 and 2.5). The calpains have been extensively studied in beef, lamb and chicken. However, there is little information on the turkey calpain proteolytic system. Previous research on turkey calpain has focused on effects of turkey age and tissue type (Northcutt et al, 1998b) and pre-slaughter stunning (Northcutt et al, 1998a) on the calpain proteolytic system. There is no available data in the literature on the changes in calpain and calpastatin during post-mortem ageing of turkey breast meat. Such information will be useful for manipulating turkey-processing procedures, to optimise calpain activity and, as a consequence, improve the tenderness of turkey breast meat.

6.1 Objectives

To investigate:

- (i) The changes in the activities of calpain I, II and calpastatin in turkey breast muscle at various post-slaughter times.
- (ii) Whether the toughness of turkey breast muscle deboned at three hours post-slaughter is associated with reduced activity of calpain.

6.2 Materials and methods

Ten turkeys of the same age (67 days old) and a mean weight of 6.2 kg were used in this experiment. The birds were processed in the same commercial turkey processing plant as in chapter 5. Each bird was electrically stunned. After stunning and exsanguination for 90 s, four birds were removed from the processing line and at-death samples taken within 5 minutes post-slaughter. The remaining six birds were processed according to the conventional method as described in section 5.1. After the first chill at 0°C for 2 h (three hours post-slaughter), the right breast muscles was removed from all six carcasses, whilst the left breast muscle remained on the bone. Then, a 3-hour sample was taken from each of the right breast muscles for tenderness determination and the pH measured. The samples were stored at -20°C until analyzed. The remaining right breast muscle and the left breast muscle on the bone was placed in individual plastic bags, and subjected to cold-blast treatment for three hours. After cold-blast treatment, all samples were vacuum-packed and aged in a chiller maintained at 4°C until 24 h post-mortem. At 24 h, the samples were taken from the processing plant to Lincoln University. At Lincoln University, the activities of calpain I, II, and calpastatin were determined. Also, turkey breast meat tenderness, MFI and sarcomere length was determined on 3-h and 24-h samples as described in chapter 3.

6.2.1 Extraction and separation of calpains and calpastatin

Calpain I, II and calpastatin were extracted using the method described by Koohmaraie (1990) with some modifications. Turkey breast samples (5 g) removed within 5 minutes of-slaughter and at three hours and 24 hours post-slaughter were homogenized in 30 ml of pre-cooled extraction buffer (100 mM Tris-HCl, 10 mM β -mercaptoethanol, 10 mM EDTA, pH 8.3) using a Polytron blender at 10,000 rpm (3x 20 s bursts, with a 20-s cooling period between bursts). The homogenate was centrifuged at 27,000 g for 30 min at 4°C (Beckman

JA-20 rotor) and the supernatant was filtered through a glass wool-cheese cloth 'sandwich'. Then, the filtrate was dialysed (12-14,000 MW cut-off) against 40 mM Tris, 3 mM EDTA, 10 mM β -mercaptoethanol, pH 7.5 for at least 12 hours and centrifuged at 27,000 g for 15 min at 4°C.

Calpains I and II, and calpastatin were separated using the method described by Morton et al (1999). The method used a 20 x 1.5 cm DEAE-Sepharose Fastflow (Pharmacia) column. The dialysed supernatant was applied to the column which was then washed with 100 ml of buffer A (40 mM Tris, 0.5 mM EDTA, 10 mM β -mercaptoethanol, pH 7.5) to remove unadsorbed proteins. Subsequently, the calpains and calpastatin were eluted at 2 ml/min by a two-stage procedure consisting of a 200 ml linear 0-175 mM NaCl gradient followed by a steeper 100 ml linear gradient of 175-500 mM NaCl. Both gradients were in buffer A.

6.2.2 Assay of calpains and calpastatin

The localisation of the fractions with active calpains and calpastatin was determined using BODIPY fluorescent microplate assay described by Thompson et al (2000). In this method, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid (BODIPYL-FL) labelled casein is used as a substrate for calpain. Sample (50 μ l) from each fraction collected was added to the wells of a black microtitre plate and 50 μ l of buffer A was added. The reaction was initiated by the addition of 75 μ l of BODIPY-FL-casein (5 μ g of Bodipy-casein/ml) in buffer A containing 10 mM CaCl_2 to each well. The microtitre plate was subsequently placed immediately in the Fluostar microplate reader equipped with PC-controlled fluorometer and the fluorescence was read at 485-nm excitation and 535-nm emission. The BODIPY-FL-casein hydrolysis was monitored every 90 s for 45 minutes. To localize calpastatin fractions, 100 μ l of m-calpain was added to all the wells and the tubes which showed inhibition were pooled and re-assayed. All fractions with calpain I and II activity were pooled separately and the activities re-assayed quantitatively using casein as a substrate, as described by Dayton et al (1976).

Aliquots (1 ml) of the pooled calpain-containing fractions were incubated at 25°C with 1 ml of 0.7% casein in 100 mM Tris-HCl, 10 mM β -mercaptoethanol, 1 mM NaN_3 , pH 7.5 and either 100 μ l of 100 mM CaCl_2 or 200 mM EDTA. The reaction was stopped after one hour

with 2 ml of 5% TCA and the mixture centrifuged at 3,000 x g for 30 min. The absorbance of the supernatant was read at 278 nm. To determine Ca^{2+} -dependent proteolytic activity, the A_{278} in the presence of EDTA was subtracted from the A_{278} in the presence of CaCl_2 . Total calpain activity was calculated by multiplying the Ca^{2+} -dependent proteolytic activity by the fraction volume and the number of pooled fractions. One unit of calpain was defined as the amount which gave a Ca^{2+} -dependent increase of 1.0 unit of absorption in one hour.

The activity of calpastatin was determined on the pooled calpastatin fractions using four tubes: a) contains calpain II + reaction mixture containing CaCl_2 ; b) calpain II + reaction mixture containing EDTA instead of CaCl_2 ; c) calpastatin extracts + 100 μl of calpain II + reaction mixture containing CaCl_2 ; and d) calpastatin alone + reaction mixture containing EDTA instead of CaCl_2 . The tubes were incubated at 25°C for one hour and the reaction was stopped with 2 ml of 5 % TCA. Then, the mixture was centrifuged at 3000 g for 30 min. The absorbance of the supernatant was read at 278 nm. Total calpastatin inhibitory activity was calculated according to the following formula: total inhibition activity = (a-b) – (c–d) x dilution factor. One unit of inhibitory activity was defined as the amount of the inhibitor that inhibits one unit of calpain II activity.

6.3 Results

6.3.1 pH and meat tenderness

Table 6.1 shows the results of the parameters measured in this experiment. At 24 hours post-mortem, the pH of the breast muscle was significantly lower than at three hours irrespective of whether the muscle was deboned at 3 hours or left 'on bone' for 24 hours. The pH of muscles deboned at three hours and then aged for 24 hours was similar to the pH of muscles left on bone for 24 hours. At 24 hours, shear force value for muscle left on bone for 24 hours was significantly lower than muscle deboned at three hours post-slaughter ($p < 0.000$). Also, the shear force value of the breast muscle at 3 hours was significantly higher than both the muscles left on bone for 24 hours and muscles deboned at 3 hours and then aged for 24 hours. This indicates that the muscles experienced pre-rigor shortening.

Table 6.1: Changes in physical and biochemical parameters associated with post-mortem storage.

Measurements	Post-slaughter Time			
	<i>At death</i>	<i>Three hours</i>	<i>'off bone' (24 h)</i>	<i>'on bone' (24 h)</i>
pH	* ^d	6.02 ^a ± 0.14	5.84 ^b ± 0.07	5.85 ^b ± 0.06
Calpain I activity	0.46 ^a ± 0.14	0.08 ^b ± 0.06	ND ^e	ND ^e
Calpain II activity	2.81 ± 0.39	2.70 ± 0.21	2.50 ± 0.27	2.57 ± 0.19
Calpastatin activity	0.64 ^a ± 0.09	0.23 ^b ± 0.09	ND ^e	ND ^e
Shear force (kgF)	*	17.3 ^a ± 2.09	12.9 ^b ± 3.60	4.83 ^c ± 1.40
MFI^f	*	20.2 ^a ± 2.31	27.0 ^a ± 4.83	50.2 ^b ± 5.69
Sarcomere length (µm)	*	1.52 ^a ± 0.13	1.78 ^b ± 0.09	1.98 ^c ± 1.01

^{abc} Means in the same row with different superscript are significantly different, $p < 0.05$.

^d Not measured, ^e Not detected, ^f Myofibrillar fragmentation index

6.3.2 Myofibrillar fragmentation index (MFI) and sarcomere length

MFI and sarcomere length of turkey breast muscle was measured at 3 hours and 24 hours post-mortem (Table 6.1). The MFI measured at 24 hours, was significantly lower for muscle deboned at 3 hours post-slaughter but aged for 24 hours compared to muscle left on bone for 24 hours. Muscles deboned at 3 hours post-slaughter and then aged for 24 hours had similar MFI values as muscles deboned at 3 hours post-slaughter but was not aged. The sarcomere length of muscle left on bone for 24 hours was significantly longer than muscle deboned at 3 hours post-slaughter and then, aged for 24 hours ($p = 0.002$). Also, the sarcomere length of muscles sampled at 3 hours post-slaughter (unaged) were significantly shorter than muscles deboned at 3 hours and aged for 24 hours ($p < 0.001$).

6.3.3 Calpains and Calpastatin

Effects of post-slaughter boning time and ageing on the activity of turkey breast muscle calpain I, II and calpastatin are shown in Table 6.1. The activity of calpain I at three hours post-slaughter was only 17% of its at-death activity. There was no detectable calpain I activity at 24 hours irrespective of whether the muscles were deboned at three hours and then, aged for 24 hours or left on bone for 24 hours. However, there was no substantial loss

in calpain II activity over the ageing period. In addition, the activity of calpain II in the muscles deboned three hours post-slaughter and muscles left on the bone for 24 hours were similar when measured at 24 hours post-mortem ($p = 0.368$). At death, calpain II activity in turkey breast muscle was four and six times more than the activities of calpain I and calpastatin respectively. Calpastatin was still active at three hours post-slaughter although only 36% of the at-death activity remains. At 24 hours, there was no detectable residual calpastatin activity in turkey breast muscle irrespective of the post-slaughter deboning time.

6.4 Discussion

The mean pH of turkey breast muscle at three hours post-slaughter (6.02) was similar to that obtained in experiment 2-4 (Chapter 5) and to the previously reported values as discussed in section 5.6.1. This experiment also confirmed that under the present processing conditions, it takes turkey breast muscle longer than 3 hours to reach ultimate pH as evident by the significantly lower muscle pH at 24 hours compared to three hours post-slaughter. Meat tenderness, MFI and sarcomere length of turkey breast muscles was determined in this section in order to evaluate the consistency of the results of chapter 5, and the relationship between these parameters and calpain activities. The result is in agreement with the results of chapter 5, which showed that at 24 hours post-mortem, meat derived from muscle deboned three hours post-slaughter but aged for 24 hours was tougher than meat from muscle aged on bone for 24 hours ($p = 0.000$). Also, sarcomere length of the muscle deboned three hours post-slaughter but aged for 24 hours, was shorter than the sarcomere length of muscle aged on bone for 24 hours ($p = 0.001$). Again, turkey breast muscle deboned at three hours post-slaughter but not aged was tougher and had shorter sarcomere length than the aged muscle. A possible reason for this observation is that the three hours muscle sample experienced severe cold shortening because the excised muscle was immediately frozen at -20°C until analysis at 24 hours post-mortem. Overall, this experiment confirmed that the deboning of turkey breast muscle at three hours post-slaughter toughens the meat.

The result of myofibrillar fragmentation index (MFI) in this experiment is consistent with that reported in section 5.5, which showed that turkey breast muscle aged on bone had higher MFI than muscle deboned three hours post-slaughter, if measured at 24 hours post-

mortem. Although low MFI indicate reduced proteolysis brought about by calpain proteolytic system, the lower MFI value in muscle deboned at three hours post-slaughter compared to muscle aged on bone for 24 hours may be attributed to the contracted state of the muscle (see section 5.6.4). The activity of calpain I in the breast muscle at death was 0.46 units/g muscle and appears to be higher than that reported by Northcutt et al (1998b), although the birds were of similar weights in both experiments. The difference between that report and the present report may be due to the buffer used to extract the enzyme. Northcutt et al (1998b) used a lower concentration of the extraction buffer than was used in this experiment. Veiseth and Koohmaraie (2001) demonstrated that extractable calpain and calpastatin activity in ovine muscle depend on the concentration of the buffer used.

While residual calpain I was detected in turkey breast muscle at death and three hours post-mortem, there was no detectable level of residual activity in muscle deboned at three hours post-slaughter and then aged for 24 hours, and muscle left on bone for 24 hours. The loss in calpain I activity was expected due to its activation and subsequent autolysis (Koohmaraie, 1990). These results confirm those of McKee et al (1997) and Walker et al (1995). These authors found that residual calpain I activity in *pectoralis* of broilers were absent at 24 hours post-mortem suggesting complete autolysis and denaturation of the enzyme. Koohmaraie (1990) demonstrated that calpain I in beef longissimus muscle retained up to 20% activity at 14 days post-mortem. Activity of calpain II and calpain I not only depends on the amount of enzyme and inhibitor present but also on muscle pH (Koohmaraie, 1990). Avian breast muscle undergoes a more rapid rate of pH fall than beef longissimus post-slaughter. Consequently, the rapid rate of pH decline in avian muscle may account for the differences in calpain I proteolytic activity in beef muscle and turkey breast muscle.

Residual activity of calpain II in turkey breast muscle at death was 2.81 units/g muscle. Northcutt et al (1998b) reported an activity of between 1.2 and 1.8 units/g muscle of a 5-week-old turkey breast whilst McKee et al (1997) reported 5.99 units/g muscle of a 48-day-old broilers. The variations in calpain II activity in these reports and the results of this experiment may be related to differences in age of the birds, species and extraction buffer. There was no significant difference between the calpain II activity at death, three hours and 24 hours post-mortem. These results confirmed Ducastaing et al (1985), Etherington et al (1987), Koohmaraie et al (1987) and McKee et al (1997) who demonstrated that normal post-mortem ageing had little effect on calpain II across species. The stability of calpain II

over the post-mortem period suggests that the intracellular level of free Ca^{2+} was too low to activate calpain II (see section 2.5). However, Walker et al (1995) observed a substantial loss in calpain II activity in broiler *Pectoralis* during post-mortem ageing, but the authors did not specify the buffer they used in their assay.

Calpastatin activity at death and three hours post-slaughter was 0.64 and 0.23 units/g muscle, respectively. This appears to be higher than the result of Northcutt et al (1998b) who reported an at-death calpastatin activity of 0.28 units/g breast muscle in a 5-week old turkey. The difference between that report and the result of this experiment may be due to the differences in strength of the buffer used in extracting the inhibitor. Calpastatin was reported to retain 20% of its at-death activity in beef longissimus muscle at 24 hours post-mortem (Koohmaraie et al, 1987). Although calpastatin was detected in turkey breast muscle at three hours post-slaughter, levels were no longer detectable in either muscles deboned at three hours and aged for 24 hours or muscles aged on the bone for 24 hours. The loss in calpastatin activity during post-mortem storage is a result of its degradation by either calpain I or other endogenous proteases (Koohmaraie, 1988; Vidalenc et al, 1983).

6.5 Conclusions

The results of this experiment agree with previous reports that calpain I and calpastatin rapidly lose their activities whilst calpain II is stable during post-mortem ageing of poultry meat. It is widely accepted that calpain proteolytic activity is responsible for meat tenderisation during post-mortem ageing (see section 2.4.3). The drastic loss in calpain I activity over the post-mortem period suggests that calpain I may be the main enzyme responsible for post-mortem meat tenderisation.

There was no evidence indicating any difference in the activity of calpain II and calpastatin in turkey breast muscle deboned at three hours and then aged for 24 hours, and muscle aged on the bone for 24 hours. Thus, the toughening of turkey breast muscle deboned three hours post-mortem is likely to be a direct result of cold shortening rather than reduced calpain activities. This suggests that the reduced proteolysis as measured by MFI is a result of reduced accessibility of the calpain to myofibrillar protein because of the shortened

sarcomere length of muscles deboned at three hours post-slaughter and then aged for 24 hours.

Chapter 7

General discussion and conclusions

This research was initially intended to study why beef of intermediate pH is sometimes tough. Preliminary sampling results (data not presented) and those reported in chapter 4 showed that it would be very difficult to obtain substantial numbers of beef samples with high ultimate pH values (> 6.0) to justify any result from such study. It was therefore, imperative to change the overall focus of the research. Hence, the main focus of this research was on the effect of post-slaughter processing conditions on turkey breast meat tenderness, which was reported in chapters 5 and 6. This research was performed for a New Zealand turkey processing company in order to identify the sources of any toughening in turkey breast meat and offer possible solutions. It was also aimed at optimising the existing turkey processing procedure in order for processors to produce consistently high quality turkey meat.

According to an unpublished turkey industry report, consumers perceived turkey meat as tough. The origin or extent of the toughness was unknown. Initially, it was difficult to know which approach would be suitable for the investigation because there was no factual evidence on the extent of turkey breast meat toughness. Furthermore, the mechanisms involved in post-mortem meat tenderisation are complex and influenced by a number of interacting factors such as processing conditions, stress, animal age and gender (Lawrie, 1992). These factors affect meat tenderness by their impact on post-mortem muscle metabolism as documented for beef or lamb (see section 2.3) Post-mortem muscle metabolism in beef or lamb is similar to that in turkey. Thus, this research was carried out using the methodology that was used to investigate the toughness of lamb and beef products.

Experiment 1 was designed to investigate whether there was any toughness in turkey breast meat as perceived by consumers. The results of this study supported the consumers' perception that turkey meat was tough in that the shear force values for some of the breast muscles were above 15 kgF even after seven days of ageing. It was shown, subsequently,

that the processing conditions affected the tenderness of turkey breast meat. For instance, blast freezing turkey carcasses increased the mean shear force or toughness of meat by 20-40%. Similarly, deboning of turkey breast muscle three hours post-mortem increased the toughness of meat by 90%. Consumer taste test data (Gilbert et al., 1990; Bickerstaffe et al, 2001) showed that consumers perceived meat with shear force value above 11 kgF as tough and unacceptable, whilst meat with shear force value less than 5 kgF and 5 – 7.9 kgF are considered very tender and tender, respectively. Based on that report, the results of this work demonstrated that turkey breast muscle aged on the bone would be tender at 24 hours and very tender by seven days. In contrast, muscle deboned at three hours post-slaughter would only be acceptable by 24 hours and tender at seven days. The toughness in turkey breast meat was found to be a result of cold shortening as evident by the shorter sarcomere length (1.49 – 1.77 μm) of muscles deboned at three hours post-slaughter compared to muscle on aged the bone for 24 h (1.90 – 2.04 μm). Muscles deboned prior to completion of rigor mortis (muscles with ATP) and exposed to cold temperature chilling, shorten because of the lack of skeletal restraint (Sams et al, 1990). Thus, it may be concluded that the completion of rigor mortis development in turkey breast muscle will require at least 3 hours under the current processing conditions. However, Alvarado and Sams (2000) suggested that deboning turkey breast at two hours post-mortem or later will not significantly impair meat tenderness.

Calpain proteolytic system is the underlying mechanism responsible for post-mortem proteolysis and meat tenderisation (Goll et al, 1998; Koohmaraie, 1996; Taylor et al, 1995; Ouali, 1990; Geesink et al, 2000). Overall, MFI values (a measure of proteolysis) at 24 hours post-mortem for meat deboned three hours post-slaughter (50-65) was lower than that for muscles aged on the bone for 24 hours (60-80). Thus, reduced proteolysis was responsible, in part, for the toughness of meat deboned three hours post-slaughter. Evidence showed that there was no significant difference between calpain proteolytic activity in muscles deboned three hours post-slaughter and muscles aged on bone for 24 hours. Therefore, it is likely that the decrease in proteolysis in muscles deboned three hours post-mortem was a result of the inaccessibility of calpain to the myofibril (due to a shortened sarcomere) rather than a decrease in calpain activity.

There is no available data in the literature on the changes in calpain and calpastatin during post-mortem ageing of turkey breast meat. It was demonstrated in this research that calpain I rapidly loses its activity during the post-mortem ageing of turkey breast meat, whilst calpain II was stable over the ageing period. The loss in calpain I activity is a result of its autolysis (Koohmaraie, 1996). Although calpastatin was detected in turkey breast muscle at three hours post-slaughter, levels were no longer detectable in either muscles deboned at three hours and aged for 24 hours or muscles aged on the bone for 24 hours. Similar results were reported for chicken breast muscles (McKee et al, 1997). The loss in calpastatin activity during post-mortem storage is a result of its degradation by either calpain I or other endogenous proteases (Koohmaraie, 1988; Vidalenc et al, 1983).

The results of this study also demonstrated that breast muscles deboned at 3 h or 24 h post-slaughter and aged for a further six days showed significant improvement in tenderness. Thus, processors deboning turkey breast muscle at three hours post-slaughter may produce consistently tender meat if the muscle is allowed to age sufficiently. In other words, ageing turkey breast muscle for 24 h prior to deboning and then ageing the muscle for a further six days will produce an excellent product.

The improvement in tenderness of turkey breast meat during the post-mortem ageing period was largely due to increased proteolysis as shown by the high MFI values and extensive degradation of troponin-T. This work confirmed that the proteins migrating at 30 kDa and 32 kDa are products of troponin-T degradation during post-mortem ageing of turkey breast meat. Previous research has shown that the 30 kDa protein is a product of troponin-T degradation and its appearance during post-mortem ageing correlates positively with meat tenderness (Ho et al, 1994).

Based on the results of this research, I therefore, proposed a new method for use in turkey processing plant (Figure 7.1). This processing method will enable turkey processors to produce consistently tender turkey breast meat.

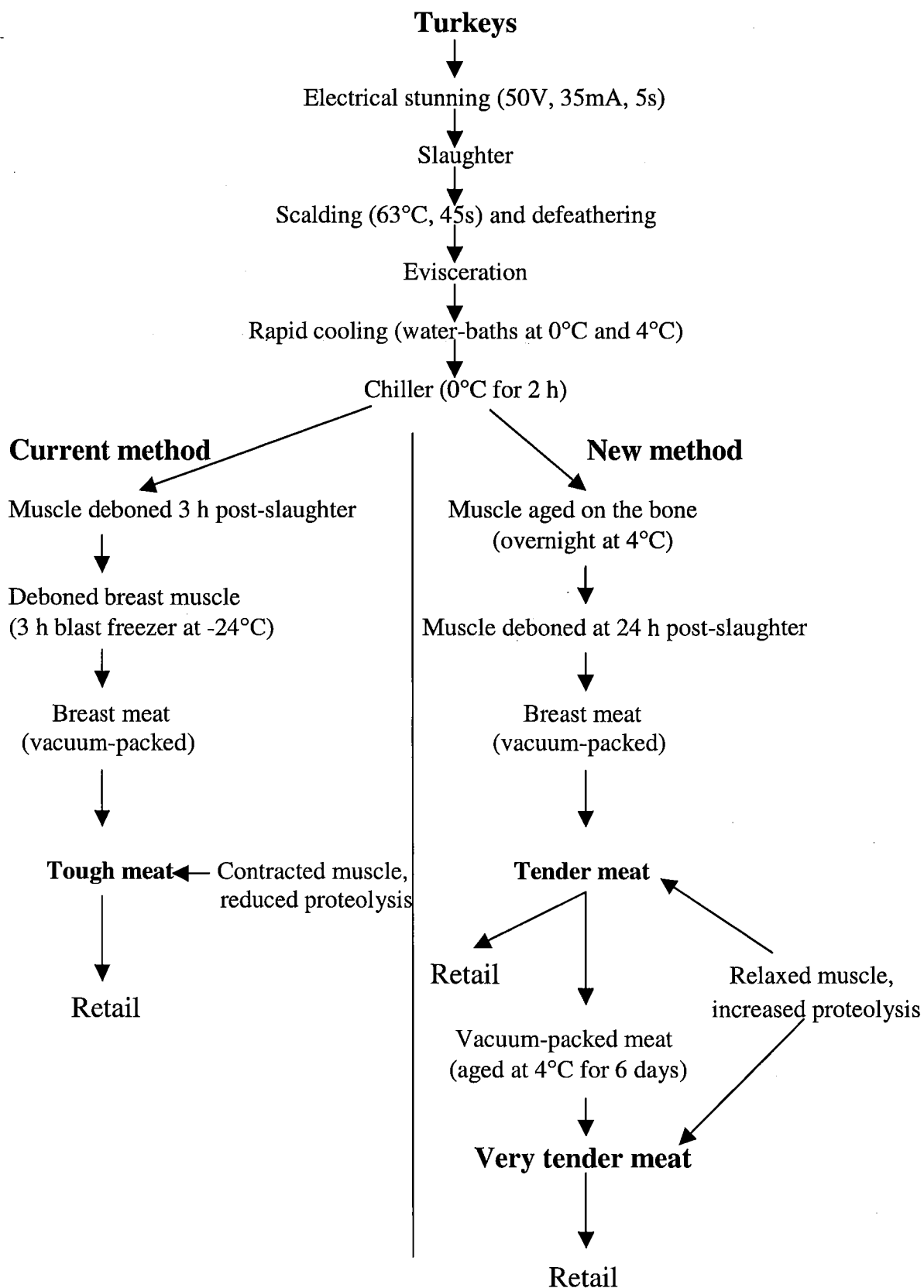


Figure 7.1: A schematic representation of the current method and proposed method (New method) of processing turkey carcasses.

In addition to post-mortem processing conditions, pre-slaughter stress has been shown to influence meat tenderness by its effects on post-mortem metabolism. The extent of tenderisation depends on the stress intensity experienced by stock. It was demonstrated in this work that spray-washing cattle has the tendency to increase beef ultimate pH and toughness. This result is consistent with previous results of the effect of pre-slaughter stress on beef quality (Beltran et al, 1997). Pre-slaughter stress should therefore be avoided if meat processors wish to reduce variability in the quality of meat.

7.1 Recommendations

Based on the results of this research, if processors wish to produce consistently tender turkey breast meat, then the existing processing procedures need to be modified (Figure 7.1). It is recommended that:

- Processors should adhere to the current bath washes at temperatures of 1°C and 4°C respectively as a means of rapidly cooling turkey carcasses. But the temperatures of the bath should be closely monitored as any variation in rate of cooling may impact on the quality of the final product.
- Carcasses should be held overnight in a chiller with a temperature range of 2°C to 5°C and then the breast muscle removed after 24 hours.
- If the processors have to debone turkey breast muscle three hours post-slaughter, then the muscle should be allowed to age for up to seven days at 4°C to produce an excellent product.
- In terms of producing turkey meat with acceptable tenderness, there is no need to use blast freezer treatment on the carcasses. This will save processors time, space, energy and cost.

7.2 Future research

- Although the effects of electrical stimulation (ES) on beef, lamb and chicken carcasses have been extensively researched, there is little information in the literature on the

effects of ES on turkey meat. Sams (1990) demonstrated that post-mortem ES prevented toughening associated with early harvesting of broiler breast fillet and reduced the variation in tenderness of the fillets. The mechanisms of ES responsible for the improvement in tenderness are the acceleration of rigor mortis development (Thompson et al, 1987) and the physical disruption of the muscle fibres (Birkhold and Sams, 1995). The available data on the effect of ES on turkey meat is inconclusive. Maki and Froning (1987) reported an accelerated muscle metabolism and reduced shear force values when turkey carcasses were stimulated with a high voltage of 800 V. The authors deboned the muscle after 24 hours post-slaughter. However, a more recent study by Owens and Sams (1997) showed that there was no significant difference in the tenderness of turkey muscles from carcasses that were stimulated with 570 V, 450 mA compared to non-stimulated carcasses, if the carcasses were deboned two hours post-slaughter. Thus, future studies on the effect of ES on turkey meat quality should be conducted to assess the effect of very early deboning times (< two hours) and the impact of different electrical parameters.

- In turkey processing, deboned breast muscles are usually treated with a blast freeze of up to -24°C in order to reduce the microbial activities and, as a consequence, improved the shelf life of the meat. However, exposing freshly deboned muscles to extreme cold temperatures of <0°C may induce cold shortening and, consequently, toughen meat as evident in experiment 4. Future research should be conducted to evaluate the extent to which the currently used blast-freeze treatment of turkey breast muscle influences the shelf life of the final meat product. It should also investigate the effects of cold blast freeze on shelf-life, tenderness and colour of the resulting meat over a range of temperatures $\leq 0^{\circ}\text{C}$. This will provide useful information on whether the blast-freeze treatment should be discontinued in any processing system.
- Further investigation into the effect of spray washing on beef quality should also be conducted. In such experiment, large number of animals should be used and the spray-washed animals slaughtered at the same time as the control animals (dry). This will enable a more conclusive evaluation of the effects of spray washing on animals.

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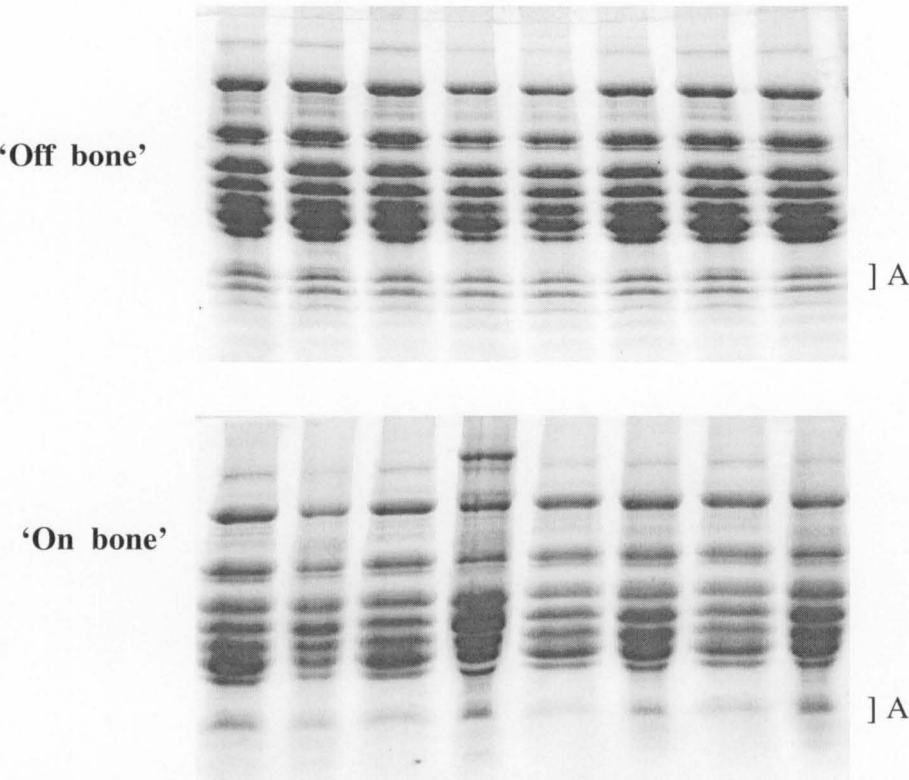
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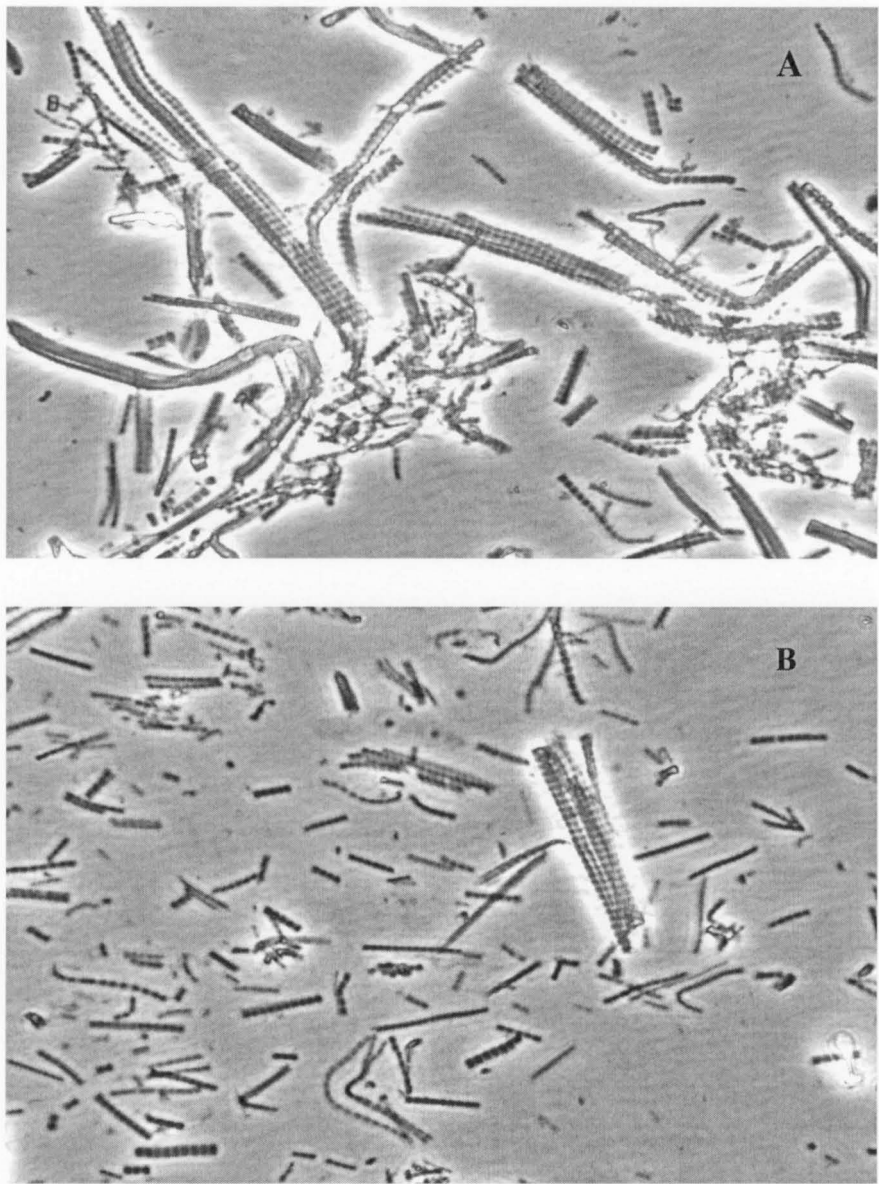
APPENDICES

APPENDIX A



Coomassie brilliant blue stained 12.5% gel of SDS-PAGE analysis of turkey breast muscle proteins from soluble fraction extract at 24h of ageing. Each lane was loaded with 40 μ g of protein in the extract. 'A' indicates the differences in the banding pattern of protein extracted from muscles deboned 3 hours post-slaughter and muscles aged on bone for 24 hours.

APPENDIX B



Fragmented myofibrils of turkey breast muscle aged on the bone for 24 hours before deboning at (A) 24 hours post-slaughter and (B) after 6 days of extended ageing (mag. x630). Note the differences in the myofibril lengths, indicating the extent of ageing.

Effect of Pre-Slaughter Stress on Beef Quality

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Keywords: stress, tenderness, pH, proteolysis, calpain

Introduction

The way an animal is treated prior to slaughter can alter the quality of the meat produced from that animal. One of the mechanisms is that pre-slaughter stress reduces the glycogen content of the muscle. These muscles produce less lactic acid post mortem and, consequently, result in meat of high ultimate pH. This meat has a dark colour, reduced flavour and is susceptible to bacterial spoilage (Lister *et al.*, 1981).

The effect of ultimate pH on the tenderness of meat is more complex. There is evidence that tenderness decreases with increasing pH up until ultimate pH's of 6.0-6.2 and then meat of higher pH is more tender (Purchas, 1990; Watanabe *et al.*, 1996).

This report considers the effect of spray washing cattle on the quality of the resulting meat. Spray washing is a hygiene requirement to reduce the contamination of carcasses but leads to a visible level of stress to the animals. Our hypothesis is that this stress will lead to meat with higher ultimate pH and altered meat quality characteristics.

Objectives

- To determine the effects of spray washing on some meat quality attributes
- To determine whether the toughness of meat of intermediate pH is a result of a shortening of the myofibrils or an effect on proteolysis (limited ageing) of the myofibrillar proteins.

Methods

A herd of cattle (Devon X) was split into two groups. The first group of 10 was held in pens without any belly wash treatment (dry pens) and the second group of 11 was held in a pen where belly sprays were turned on every hour for five minutes. Animals from the dry pens were killed first whilst the spray-washed animals were killed later in the day.

The following procedures were performed on each longissimus dorsi (LD) samples from the two groups of carcasses: measurement of ultimate pH, shear force determination at 24 hours post mortem, myofibril isolation and myofibril fragmentation index (MFI) determination at 24 hours and 7 days, sarcomere length measurement using phase contrast microscopy and Western blotting of μ -calpain, troponin T and desmin (Geesink *et al.*, 2001).

Results and Discussion

The LD muscles from the spray-washed and dry animals were analysed for their ultimate pH and shear force at 24 hours. Spray washing resulted in considerably more variation in the pH of the meat (5.52 to 6.48) compared to meat from the dry animals (5.50 to 5.79). The increased variability in pH was reflected in the meat shear force values from the spray-washed animals which ranged from 5.2 to 14.5 kgF while that from the dry animals ranged between 4.8 and 8.6. The mean pH and the shear force of the meat from the spray-washed animals were higher but the difference was not significant ($p=0.09$, Table 1). This is probably due to the high variability of the measured meat parameters from the spray-washed animals.

The LD samples were further analysed to determine the cause of any toughening. Two factors which can contribute to meat toughness are the state of contraction of the muscle (measured as sarcomere length) and the extent of any post-mortem proteolysis (measured as myofibrillar fragmentation index). The LD's from spray washed animals had shorter sarcomeres than those from dry animals ($p = 0.069$). This is one possible reason for the slightly increased meat toughness associated with the spray washed animals. However the myofibrillar fragmentation index measured at one ($p=0.67$) and seven days ($p=0.40$) post mortem were essentially the same for both spray washed and non-sprayed animals. Thus, there was no evidence that spray washing contributed to the toughening of LD by reducing protein breakdown.

There have been many reports of a relationship between ultimate pH and shear force. In this study the pH was positively correlated with the shear force at 24 hours of ageing ($r = 0.760$). However as shown in Fig.1 the relationship was not linear. The shear force increased as the ultimate pH progressed from 5.6-5.9 and peaked between pH 5.9 and 6.2. As the pH increased above 6.2 the shear force tended to decrease. The results fitted a curvilinear relationship as found in earlier research (Purchas, 1990).

The results of this experiment indicate that ultimate pH is inversely related to the sarcomere lengths ($r = -0.525$). The shortest sarcomere length (1.49 μ m) was at an ultimate pH of 5.79. The sarcomere length decreased linearly as the ultimate pH moved towards an intermediate pH range (5.8-6.2). Thereafter, the sarcomere length increased as the ultimate pH increased. This curve is an inverse of that for pH which suggests that the shorter sarcomere lengths may be a cause of the increased shear force (Fig.1).

The ultimate pH could also alter the shear force by its effect on the activity of proteolytic enzymes. The result of proteolysis can be measured by myofibrillar fragmentation index (MFI). After one day of ageing, there was no evidence of a relationship between MFI and ultimate pH although it appears that low ultimate pH (5.47) or high ultimate pH (6.48) meat samples have high MFI values. Post-mortem proteolysis is generally considered to be due to the action of μ -calpain (Koochmarai, 1996; Geesink *et al.*, 2000). Once μ -calpain is activated it not only degrades other proteins but also it degrades itself, a process named autolysis. Thus it is possible to

measure the activity of the calpains by determining the extent of μ -calpain autolysis. Western blotting of μ -calpain showed that the extent of autolysis at 24 hours was not related to the MFI at day 1 but was related to the MFI at day 7. There was no evidence of an effect of spray washing on μ -calpain autolysis. Western blotting of two muscle proteins that are substrates for calpain revealed a relationship with tenderness. Troponin T degradation was negatively correlated with pH and shear force. That is, the tough carcasses were those with the least degraded troponin T. However the spray washing had no effect on degradation of troponin T. Desmin was extensively degraded in the seven days post-mortem. The extent of this degradation was related to the MFI and there was a trend ($p = 0.079$) towards less degradation in the meat from those animals which had been spray washed.

Conclusions

- The limited size of the trial and variability of the meat from spray washed animals prevented any significant results being obtained. However there was a trend towards spray washing causing a rise in ultimate pH and a toughening of the meat. This is more likely to be related to shorter sarcomeres than reduced proteolysis.
- The shear force increased as the ultimate pH reached 6.2 and then decreased.
- Sarcomere length decreased until pH 6.2-6.3 and then increased in a mirror image of shear force.
- Proteolysis, as measured by MFI and Western blotting, increased with pH. Note, however, that although the mean MFI values at pH 5.5 to 5.7 were low, the highest MFI values were from this group.

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Table 1. Effect of spray washing on LD ultimate pH, shear force at 24 hours, MFI at 24 hours, sarcomere length, autolysis of μ -calpain and degradation of tropinin-T and desmin. Autolysis of μ -calpain was determined by the ratio of the density of the higher molecular weight band for the large subunit to the density of the lower molecular weight band. Degradation of tropinin-T and desmin was determined by the relative density of the bands at day 7 and day 1. The values shown are means followed by the standard error of the mean.

	Number of samples	pH	Shear force (kgF)	MFI (1 day)	Sarcomere length (μ m)	μ -calpain	Tropinin-T	Desmin
Control	10	5.58 (0.03)	6.3 (0.37)	68 (4.9)	1.86 (0.04)	1.81 (0.13)	0.74 (0.04)	0.03(0.02)
Washed	11	5.77 (0.12)	8.1 (0.89)	71 (2.7)	1.73 (0.05)	2.42 (0.63)	0.76 (0.10)	0.17(0.07)
t-test (p)		0.14	0.09	0.67	0.07	0.37	0.88	0.079

Figure 1. The relationship of LD ultimate pH with shear force at 24 hours post mortem (Δ) and sarcomere length (\blacksquare).

